

CFTRI-MYSORE



3304

Physiology and b.

1



2000
10
PIC
1000

1000

1000

0-4 DUE

Page No.	Date of Issue	Date of Return
28.1.1956		
196	22-1-56	G-2
71	1-7-57	L

7

7

7

7.11.1956
VENKATADRIKALU POND
VENKATADRIKALU POND
VENKATADRIKALU POND

*Physiology and Biochemistry
of the Skin*

LIST OF CONTRIBUTORS

ZACHARY FELSHER, B.A., M.D.

*Instructor of Dermatology
Rush Division, University of Illinois*

PETER FLESCH, M.D., PH.D.

*Assistant Professor of Research in Dermatology
University of Pennsylvania*

AARON BUNSEN LERNER, M.D., PH.D.

*Associate Professor of Dermatology
University of Oregon*

ALLAN L. LORINCZ, M.D.

*Assistant Professor of Dermatology
University of Chicago*

HERMANN PINKUS, M.D.

*Clinical Associate Professor of Dermatology
Wayne University*

GEORGE C. WELLS, M.B., M.R.C.P.

*Assistant Professor of Dermatology
University of Chicago*

Physiology and Biochemistry of the Skin

By

STEPHEN ROTHMAN, M.D.

Professor of Dermatology, University of Chicago



THE UNIVERSITY OF CHICAGO PRESS

THE UNIVERSITY OF CHICAGO COMMITTEE
ON PUBLICATIONS IN BIOLOGY AND MEDICINE

EMMET B. BAY • LOWELL T. COGGESHALL
LESTER R. DRAGSTEDT • FRANKLIN C. McLEAN
THOMAS PARK • WILLIAM H. TALIAFERRO

3304

L, 87:3

N54

CFTRI-MYSORE



3304

Physiology and b...

THE UNIVERSITY OF CHICAGO PRESS, CHICAGO 37
Cambridge University Press, London, N.W. 1, England
The University of Toronto Press, Toronto 5, Canada

Copyright 1954 by The University of Chicago. All rights reserved. Published 1954. Composed and printed by THE UNIVERSITY OF CHICAGO PRESS, Chicago, Illinois, U.S.A.

No illustration or any part of the text may be reproduced without permission of The University of Chicago Press

*This book is dedicated
to the memory of my teacher*

ALBERT JESIONEK

1870-1934

Foreword

THE main purpose of this book is to serve dermatological research. A review of present knowledge in the field of the physiology and biochemistry of the skin may aid dermatological investigation, because thorough knowledge of morbid processes is inconceivable without an understanding of the functions which are disturbed. For instance, we cannot understand seborrhea or acne vulgaris without studying the normal function of sebaceous glands, or keratinization anomalies without investigating the biological processes in the normal course of keratinization, or the influence of emotional factors on skin diseases without studying the physiological nerve impulses running from the cerebral cortex to the skin.

Basic research in dermatology is a young branch of the clinical sciences. I have witnessed its rapid development during the last twenty years. When, about twenty-five years ago, I first attempted to review all available data on the biochemistry of the skin, my bibliographic studies often were frustrating because of scantiness of pertinent contributions. It was not much better five years later. By now the situation has become reversed. The material has become overwhelmingly large. In preparing this book, particularly during the last two years, I opened with misgivings and uneasiness the newly arriving issues of biomedical periodicals, because their appearance often meant that additions and corrections had to be made in chapters which were by then "completed."

The material which pertains to the physiology and biochemistry of the skin in one way or another is so immense that it was obviously impossible to review it in one volume. Some kind of selection had to be made. One principle of selection was to deal in greater detail with functions of the skin

that may be considered unique, such as sweat secretion, keratinization, sebum formation, and melanin production. More general functions, in which the skin participates in some particular way, such as cutaneous circulation and vascular reactions, are also treated in detail. The other principle of selection was, corresponding with the purpose of the book, to put emphasis on facts which seem to be most important for basic research in dermatology. For instance, among sensory functions, only perception of pain and itching are discussed in any considerable detail. Or thermoregulation is dealt with only from the viewpoint of investigational dermatology. Further, the biochemistry of nucleoproteins is not discussed at all, because, so far, they have not been shown to behave differently in the skin from the way they do in other organs.

In addition to these legitimate principles of selection, I have, no doubt, allowed personal bias to dictate arbitrary choices or emphasis. I have discussed in greater detail problems on which I myself had been working and those which in my personal opinion seemed most important. Of course, any piece of basic research, no matter how insignificant it may appear at the moment, may one day prove to be fundamental for clinical application, and nobody, including myself, can predict whether or not an experiment will be fruitful in the future.

Beyond these selections, whether objectively justified or merely arbitrary, there obviously is a number of conscious and unintentional omissions. For example, the function of the skin as an excretory organ for foreign substances (with the exception of hair) has not been dealt with, because it does not belong strictly to physiology and biochemistry and would have added unduly to the length of the volume. The physiology of nail formation and nail growth has not

been discussed, because the available data are too scanty for a comprehensive presentation. Quite a few worthy contributions, valuable in themselves or for their possible applicability in clinical research, have not been mentioned, because everything could not be included. As to the unintentional omissions, regrettable as it may be, they are likely to occur in any attempt to make a comprehensive survey of the world literature of the last twenty-five years.

I have attempted to establish a proper basic level of discussion and to maintain it consistently. Ideally, the book should have been written for readers who know as much physiology and biochemistry as is taught in college and medical school. However, too many medical men have forgotten their college physiology and biochemistry, and, among those who have not, too few have kept abreast of new developments in the field. In general, I have tried to avoid recapitulation of facts that can be found in current texts of physiology and biochemistry, with the exception of some general remarks on proteins, enzymes, and vitamins which were included because of their paramount significance.

Discretion had to be observed in regard to the application of physiological knowledge to problems of pathology and clinical medicine. On the one hand, there was the danger of excessive reference, with the end-result of unwittingly turning a book on the physiology of the skin into a text on clinical dermatology. On the other hand, some applications to clinical medicine were important enough to be mentioned. An effort was made to limit such discussions to immediate, hitherto unbroached, problems which can be approached easily by simple experimental arrangements. I have tried to avoid discussion of morphological details, because many good texts are available on the morphology of the skin. Description of methods was entirely omitted because it is beyond the scope of the book.

In organizing the contents, I found it advantageous not to separate strictly chapters on physiology from chapters on bio-

chemistry, because physiological functions and biochemical processes are greatly interdependent. I discuss the composition of sweat and the biochemistry of the aqueous surface film right after discussing the physiology of sweat secretion and, similarly, the biochemistry of the lipid surface film right after dealing with the physiology of sebaceous excretion, because most constituents of the aqueous film stem from sweat-gland activity and most constituents of the lipid film from sebaceous-gland activity, even though keratinization also participates in producing these films. The biochemistry and metabolism of the main constituents of skin tissue—proteins, carbohydrates, lipids, water, electrolytes, and enzyme systems—are dealt with in subsequent chapters. But even these apparently purely biochemical chapters contain many physiological data, as, for instance, all the chapter on keratinization.

Criticism could be made of my excessively historical approach to the knowledge in some fields or, colloquially, of my handling too much "old stuff." If the work is criticized on this ground, I can merely reply not only that in this matter I have followed my personal inclination but also that such an approach has a certain didactic value. We live in an age of rediscoveries. And, also, many young investigators imagine that the seemingly miraculous development of the medical sciences in the last two decades came about without any preliminaries by virtue of the excellence of this generation alone—somehow as Pallas Athene sprang forth fully armed from the skull of her father Zeus. At times I have tried to point out the fallacy of this naïve belief and to stress the gradual, almost painful, course of development through devious and erroneous pathways.

As a result of this pursuit, it is possible that I have neglected some of the most recent publications. I must take the blame for this shortcoming and can only say to any criticism of it that I preferred to report on older confirmed facts rather than on new unconfirmed ones. Very recently, a similar

attitude was expressed by W. H. Hay in the *American Scientist*, with a quotation from George Sarton: "Knowledge of the past will bring out the distinctive characteristics of the whole process, giving us 'a less conceited view of our share, since the whole of past history is there to testify that contemporary judgments are always precarious.' " On the other hand, by incorporating everything worth while published up to the time this work went to press, my young contributors have undoubtedly done much better than I, not being handicapped by conservatism.

Among the contributors, Drs. Felsher, Flesch, Lorincz, and Wells are now or were in the past members of the staff at the University of Chicago. I was also privileged to

follow closely the distinguished careers of Drs. Lerner and Pinkus. The participation of these contributors has not altered the planned homogeneity of this monograph. They certainly took a considerable load off the shoulders of the senior author when they graciously and unselfishly consented to help him write this book.

If this book awakens some interest for basic research in young dermatologists and helps and strengthens the enthusiasm of the few dozen young men who already have devoted themselves to investigative dermatology, my labors and those of my colleagues will be richly compensated.

STEPHEN ROTHMAN

CHICAGO
May 1953

Acknowledgments

WHEN fifteen years ago I came to the United States, I had the good fortune to become associated with the University of Chicago. There I experienced such measure of freedom as is undreamed of in those parts of Central Europe where I had worked before. Therefore, my first heartfelt acknowledgment must go to the University of Chicago. This institution made it possible for me and my associates to pursue energetically a great number of problems in the field of investigative dermatology.

From the beginning I had wonderful support, encouragement, and assistance from the leaders of investigative dermatology in this country: Dr. Donald M. Pillsbury, University of Pennsylvania; Dr. Marion B. Sulzberger, New York University; and the late Dr. J. Gardner Hopkins, of Columbia University.

I was received, encouraged, and helped with the same kindness and friendliness in the Chicago Dermatological Society by the top representatives of dermatology in the Middle West: Dr. Francis M. Seneor, University of Illinois; Dr. Henry E. Michelson, University of Minnesota; Dr. Paul A. O'Leary, Mayo Clinic; Dr. Arthur C. Curtis, University of Michigan; Dr. S. William Becker, University of Chicago, and others.

In preparing this book all the research dermatologists in this country helped me. Particular thanks are due to Dr. Raymond R. Suskind, University of Cincinnati, who put at my disposal his unparalleled bibliography. I am grateful to Drs. Irvin H. Blank, Harvard University; Eugene M. Farber, Stanford University; Peter Flesch, University of Pennsylvania; Franz Herrmann, New York University; Walter C. Lobitz, Jr., Dartmouth Medical School; Allan L. Lorincz, University of Chicago; William Montagna, Brown University; J. P. O'Brien, University of Sydney; and Walter B. Shelley, University of Pennsylvania. These

men sent me their unpublished manuscripts, discussed with me their current work, and went over large parts of my manuscript. Mrs. Margaret Gomori, librarian in Billings Hospital, University of Chicago, gave me the best possible service with kindness and great efficiency. Dr. Carl T. Nelson, Columbia University, most kindly helped me in my bibliographic studies in New York.

I am indebted to Drs. Konrad Bloch, Lillian Eichelberger, Nathan Kleitman, Albert L. Lehninger, all of the University of Chicago, and also Dr. Sheldon A. Walker, my former associate, for reading and helping to correct several chapters of the book.

Miss Grace Knox, member of the staff of our Section of Dermatology, revised the manuscript and prepared the picture material. Miss Knox worked with genuine devotion, sacrificing all her free time for many months. My wife, Dr. Irene Rothman, not only helped with the reference work but also put up patiently with a distraught husband, who, on top of his full-time job, tried to write a book. The faithful and devoted secretary of the department, Mrs. Myrtle Olson, most efficiently handled all organizational work. Mr. Norman Britan, University of Chicago, gave valuable assistance in reference work.

This work is dedicated to the memory of my teacher, Albert Jesionek, professor of dermatology at the University of Giessen, who was a fatherly friend to me. He was one of the early pioneers who had the vision and courage to break away from the purely descriptive dermatology of the last century. Both his monographs on *Photobiology* (1912) and on *Biology of the Normal and Diseased Skin* (1916) bear witness to his dynamic thinking.

I also remember with affectionate gratitude and reverence my early teachers in biochemistry and physiology, Franz Hofmeister, Karl Spiro, and Franz Tangl, all representatives of a great tradition,

Table of Contents

1. MECHANICAL PROPERTIES	1
2. ELECTRICAL BEHAVIOR	9
3. PERCUTANEOUS ABSORPTION	26
4. CIRCULATION AND VASCULAR REACTIONS	60
5. SENSORY FUNCTIONS	120
6. SWEAT SECRETION	153
7. COMPOSITION OF ECCRINE SWEAT AND AQUEOUS SURFACE FILM	201
8. pH OF SWEAT AND SKIN SURFACE	221
9. INSENSIBLE WATER LOSS	233
10. THE ROLE OF SKIN IN THERMOREGULATION	244
11. THE SWEAT-RETENTION SYNDROME	270
12. SEBACEOUS-GLAND EXCRETION	284
13. COMPOSITION OF THE LIPID SURFACE FILM	309
14. NITROGENOUS CONSTITUENTS; GENERAL DATA	337
15. EPIDERMAL PROTEINS	343
16. THE KERATINIZATION PROCESS	366
17. COLLAGEN, RETICULIN, AND ELASTIN <i>Zachary Felsher</i>	391
18. CONNECTIVE-TISSUE GROUND SUBSTANCE <i>George C. Wells</i>	418
19. CARBOHYDRATES	465
20. LIPID CONSTITUENTS	483
21. WATER AND ELECTROLYTES	493
22. PIGMENTATION <i>Allan L. Lorincz</i>	515
23. ENZYMES <i>Aaron Bunsen Lerner</i>	564
24. CARBON DIOXIDE DELIVERY	580
25. BIOLOGY OF EPIDERMAL CELLS <i>Hermann Pinkus</i>	584
26. HAIR GROWTH <i>Peter Flesch</i>	601
27. NUTRITIONAL INFLUENCES <i>Allan L. Lorincz</i>	662
28. PATHOPHYSIOLOGY OF BLISTER FORMATION	699
SUBJECT INDEX	709
AUTHOR INDEX	720

CHAPTER I

Mechanical Properties

I. TENSION	1
II. RESILIENCY	3
III. TENSILE STRENGTH	5

I. TENSION

IF A piece of skin is cut out of its natural habitat, its edges will retract, a sign that in vivo the skin is stretched to some degree, like a somewhat tight glove which is forced on the hand. Because of this original tension, stretching by weak forces in vivo is

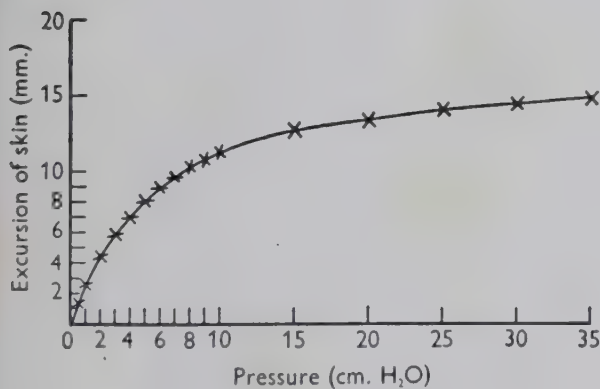


FIG. 1.—Graph of excursion of skin against pressure. Male, twenty-five years. Skin of thigh, medial side. From Dick (6). (Reproduced by permission of the University of Cambridge Press.)

resisted, and only greater forces will be effective in expanding the skin (6).¹ In the work of J. L. Dick (6) the initial resistance to quantitatively graded deforming forces has been taken as a measure of the tension. By measuring the maximum deforming force which is resisted by the skin, the tension

1. Numbers in parentheses indicate references cited at end of each chapter.

can be expressed in dynes per centimeter (6). The tension is greater in areas supplied by dense elastic fiber nets, and particularly in regions where the skin is thin and at the same time rich in elastic fibers, such as the tibial region. It is less in areas where the skin is relatively thick, e.g., the epigastrium. The tension is much greater in the skin of the young than in that of the old (Figs. 1 and 2). Correspondingly, shrinkage of excised pieces of skin is much greater in young adults than in old people (1, 8). But it is not so great in childhood as it is in early adulthood (1).

The tension has been explained by the presence of abundant, somewhat stretched, elastic fibers. When the skin is placed under increasing tension, first the elastic fibers and then the collagen fibers become stretched. Old people's skin, with poor elastic fiber supply, has a low initial tension and shows little resistance to slight stretching, but it expands relatively less than the skin of the young when the force is increased and collagen fibers are stretched (6) (Fig. 2).

Membranes inside the body, such as the fascia lata or the dura mater, have no initial tension. Like rubber, they expand greatly under the first few increments of stretching force, but the expansion gradually diminishes with further increments of the force (6).

The direction of the tension of the skin is

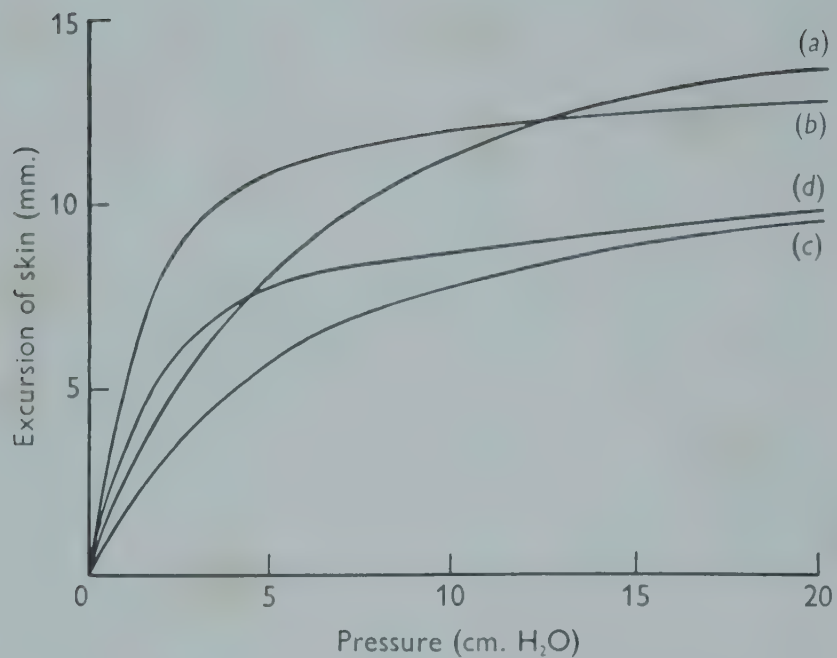


FIG. 2.—Graphs of excursion of skin (mm.) against pressure (cm. H₂O) for the medial sides of the thigh and of the leg, from a young (twenty-five-year) and from an old (sixty-five-year) male. (a) Thigh, medial (twenty-five years); (b) thigh, medial (sixty-five years); (c) leg, medial (twenty-five years); (d) leg, medial (sixty-five years). From Dick (6). (Reproduced by permission of the University of Cambridge Press.)



FIG. 3.—Cutis verticis gyrata

arranged in an elaborate line system. Almost a hundred years ago K. Langer (17) studied these directions all over the body surface by puncturing the skin of cadavers with a round awl. The round defect, due to the uneven tension, was drawn into a longitudinal slit along the direction of greater tension. These "cleavage lines" lie in the direction of lesser extensibility and perpendicular to the direction in which the skin can be stretched most (19). When the volume of the abdomen or of the extremities increases, the overlying skin stretches with ease in this perpendicular direction; but undue stretching in the direction of the cleavage lines causes rupture of fibers and formation of striae distensae (19). Striae can be formed in normal skin when the stretching force is unduly high, but they are most commonly seen if, in addition to stretching, there is abnormally low tensile strength of the skin, as is the case in pregnancy and in Cushing's syndrome.

Whereas the skin normally covers the underlying tissues tightly under some tension, it may under certain circumstances

become loose and redundant, spontaneously forming folds. The skin of the scalp, for instance, might be disproportionately large for the skull it covered, in which case the characteristic cutis verticis gyrata of Jadasohn (12) develops (Fig. 3). These and similar anomalies were discussed by F. Pinkus (19) under the heading of "developmental folds." Another cause of fold formation can be diminution in the volume of the fatty panniculus without volume changes in the skin. The disproportion between these two volumes can be exaggerated by an increased water content of the skin, as is the case in infants with alimentary disturbances showing a characteristic wrinkled "senile" skin. Finally, the original tension of the skin may be relaxed because of degeneration of its elastic fibers, which are primarily responsible for this tension. In this case the skin again becomes too "wide" for the area it is supposed to cover tightly, and wrinkles develop for this reason. They are characteristic of senile skin. The "cigarette paper wrinkling" in acrodermatitis atrophicans is a special case of such loosening.

II. RESILIENCY

Qualitatively, elasticity is usually defined as the ability of materials to resume their original shape after external forces have caused a deformation and then ceased to act. By this definition the skin *in vivo* is an "elastic" body. Because this "elasticity" cannot be quantitatively determined, it is probably preferable to talk of "resiliency."

Many attempts have been made to measure this resiliency *in vivo*. With minor modifications, the "elastometer" of Schade (21, 22) is still being used for this purpose. The instrument measures and registers the indentation of the skin under the pressure of graded weights and the return of the indented skin to its normal position after removal of the weights. A modified "elastometer" was recently developed by Kirk (16) and is described in detail by Kirk and Kvorning (15). Edematous skin treated in this way shows much slower deformation and

slower return to the original state than does normal skin (21).

Kirk and Kvorning (15) found distinctly different "elastic" behavior of the skin according to age. In young adults the immediate indentation was an average 82 per cent of the total (more slowly completed) indentation, while in the aged this average percentage was 70. The total indentation was greater in the young than in the old (3.4 mm. versus 2.7 mm.). Even greater were the differences in the rebound. The immediate rebound was an average of 76 per cent of the indentation in the young, 43 per cent in the old. In the light of these measurements the "elasticity" of the skin was not perfect *in vivo*. At the end of their experiments Kirk and Kvorning found 92 per cent rebound in the young and 81 per cent in the old. In strips of cadaver skin the return to the original length after stretch-

ing is always incomplete to a degree, depending not only on the absolute amount of force but also on the rapidity with which the stretching force is applied (25).

Measurements with the modified "elastometer" indicated a considerable "rejuvenation" of senile skin in men and women after systemic and/or topical applications of estrogenic hormones (4). Topical treatment with estrogens was claimed to improve the resiliency of old people's skin to a degree that it corresponded to a rejuvenation of about 15 years (4). The same treatment was found to effect a redevelopment of elastic fibers, disappearance of collagen fragmentation, increased number of capillary blood vessels, and increase in the number of epidermal cell layers (11, 9, 10).

Should these findings be confirmed, it would appear that physical measurements and histological findings can be related: degeneration of elastic and collagen fibers, changes in the ground substance (18), poor blood supply, and thinning of the epidermis in senescence are accompanied by decreased resiliency, and both can be reversed by local applications of estrogens. The effect of locally applied male sex hormone was found to be less pronounced than that of estrogens (10).

A different method of estimating resiliency of the skin was introduced by Jochims (13). First, he measured the force required to compress maximally a skin fold of a certain thickness. Then, gradually releasing the external force, he estimated at what point of decreasing force there was a smoothing-out of the fold. In normal infants he found the force required to compress the fold maximally to be about twice as great as the force at which the skin retracts from the folded state. He interpreted this observation by assuming that the external force has to overcome an elastic and a nonelastic resistance of the skin. Elastic resistance is the force at which the folding is overcome. Later on (14), this view was somewhat modified.

In any case, Jochims' measurements on diseased children (13) revealed that the skin of infants with malnutrition (so-called

"dystrophy") is characterized by (a) formation of unusually thin folds and (b) greatly decreased tendency to retract from the folded state. In contrast, the skin of infants suffering from acute diarrhea, dehydration, and acidosis (so-called "alimentary toxicosis") forms normally thick folds, requires normal force to make and compress a fold, but shows a decreased tendency to retract the fold when the force is released.

According to Jochims (13), one component of elastic skin resistance is its water content. With loss of water, this resistance decreases. What is commonly called "turgor" of tissues is probably this component. It is common knowledge that, in states connected with dehydration, folds formed by lifting the skin smooth out more slowly than normal.

A somewhat modified way to measure the tension, extensibility, and contracting tendency of the skin has been the measurement of the necessary diminution of the distance between two parallel lines drawn on the skin to form a fold (14, 7). The two lines are brought closer to each other by pressure from the sides with an instrument (14) or with the fingers (7). The "contractility" of the skin is expressed by the formula $C = (L - L_0)/L$, where L is the original distance between the two parallel lines and L_0 is the distance at the time when a fold has just been produced (7). The closer one can bring the two lines without fold formation, the greater is the "contractility." This "contractility" seems to be analogous to the "original tension" as defined by Dick (6). In infants suffering from malnutrition (so-called "dystrophy") it was found to be greatly reduced.

Folds produced by lifting or by compression may be folds of the skin proper or folds including the subcutaneous fat tissue. Thickness of folds of the latter variety have proved to be good indices of nutritional state and water content (23, 2, 3).

Tui *et al.* (24) described a "tissue tonometer," in principle the same as the tonometer of ophthalmologists measuring the displacement (or "depression") of the

skin under given weights. With this instrument the authors were able to follow the soft-

ening of sclerodermatous skin under the influence of therapeutic measures.

III. TENSILE STRENGTH

The average tensile strength of the skin of human cadavers (without subcutaneous fat) was found to be 1.8 kg/sq mm (26). However, the average value varies greatly with age (20). In infants up to three months of age, it is only 0.25–0.30 kg/sq mm; in children three months to three years old, it is 0.53–1.4 kg/sq mm. In adults from fifteen to fifty years old, the average is 1.61 ± 0.08 kg/sq mm; and in old persons from

The increasing resistance to stretching with advancing age is best illustrated by calculating the modulus of elasticity, which is expressed as the quotient stress/strain or force/deformation. The less the deformation under a given force or the greater the force required to produce a certain deformation, the larger is the modulus of elasticity.

Rollhäuser (20) gives the data shown in Table 1. This spectacular increase of the

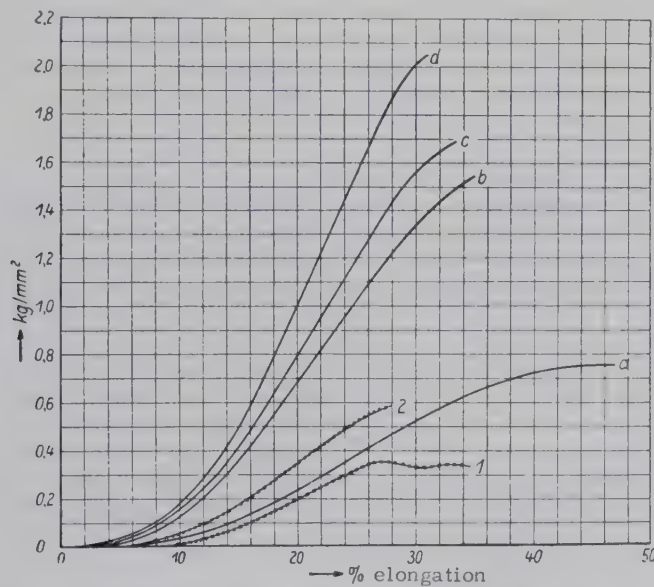


FIG. 4.—Extensibility curves of the skin. Average values for different age groups. (a) Children, from infants born two months prematurely to the age of three years; (b) fifteen to thirty years; (c) thirty to fifty years; (d) fifty to eighty years. Curves 1 and 2 are from cases with unusually weak skin. From Rollhäuser (20). (Reproduced by permission of Akad. Verlag Geest & Portig K.G.).

fifty to eighty years old, 2.05 ± 0.11 kg/sq mm (20).

Not only the resistance of the skin to tearing under tension but also its extensibility under tension are functions of age (Fig. 4). The older the individual, the less extensible is his skin. In the newborn an excised skin strip can be elongated by 50–59 per cent of its original length before it tears. In infants this extensibility varies between 37 and 52 per cent, in adults between 24 and 48 per cent. Within the adult group, however, the degree of elongation before tearing is not clearly dependent on age (20).

modulus of elasticity with age means an increased resistance to stretch and/or a decrease in extensibility.

In his material Rollhäuser (20) found a few samples with abnormally low tensile strength, deriving mainly from adult patients who had died from some consumptive disease. But the low values were not characteristic of any particular disease. The histological picture in these cases showed little deviation from normal, if any. Possibly the collagen fibers were more split than normal, and there was less intertwining of fibers. By far the lowest values were found in a case

of Ehlers-Danlos syndrome (cutis laxa). The tensile strength in this thirty-five-year-old male's case was 0.34 kg/sq mm. Figure 5 shows one of my own cases of cutis laxa.

In the interpretation of Rollhäuser (20) the tensile strength of a heterogeneous material, such as the skin, depends on qualita-

compression. As stretching progresses, there is a jamming of fibers, whereby the resistance to stretch increases. A dense network of fibers with strong anchoring between them has a greater tensile strength than a loose network in which fibers may glide along one another.

TABLE 1
AVERAGE MODULUS OF ELASTICITY OF THE HUMAN SKIN
AT DIFFERENT AGE GROUPS (20)

	AGE GROUP			
	7 Mo.-3 Years	15-30 Years	30-50 Years	50-80 Years
Modulus.....	2.9 ± 0.45	6.7 ± 0.29	8.1 ± 0.34	11.0 ± 0.47



FIG. 5. Cutis laxa (Ehlers-Danlos syndrome)

tive as well as quantitative factors, viz., on the mechanical properties of its strongest element, on the relative amount of this element, and on its texture. Energetic stretching of the skin leads to deformation of the collagen bundles, and the angles of fiber-crossing change. Initially, there is little resistance to stretch because for a while the stretched fibers are not jammed by cross-

Another important factor is the water content. It influences tensile strength in two ways: first, fluids have no tensile strength, and therefore the greater the water content, the smaller the tensile strength; second, water facilitates the slipping of fibers along one another and thereby further decreases tensile strength (20). In this interpretation the low tensile strength of infan-

tile skin is caused by its high water content and by the looseness of its collagen network. With advancing age the fibers become progressively matted and intertwined until compact bundles are formed in the adult.

Rollhäuser (20) found that individual differences in tensile strength of the skin are paralleled by similar differences in the tendons. Therefore, he believes that tensile strength is a systemic (constitutional) property of the individual's fibrillary structures. Wenzel (25), using similar methods, arrived at results similar to those of Rollhäuser. He found the greatest tensile strength and greatest modulus of elasticity always along the main direction of the collagen fibers.

While Rollhäuser found no appreciable sex differences, Wenzel's measurements (25) showed less tensile strength in female skin. His most important findings concern the skin in pregnancy and in Cushing's syn-

drome. In pregnant women and even more in patients with Cushing's syndrome the tensile strength of the skin is as low as in infants or lower. It has a similar great extensibility and similar low modulus of elasticity. Conversely, Wenzel (25) finds surgical (fibrous) scars less extensible than normal skin, but with a low modulus of elasticity and somewhat decreased tensile strength.

Interestingly, Wöhlisch (26) found that the tensile strength of the epidermis + corium is greater than that of the corium alone. Thus, apparently, the epidermis has the greater appreciable tensile strength. However, Rollhäuser (20), controlling the events after stretching microscopically, saw tears in the epidermis much earlier than in the corium. No data could be found on the tensile strength of the skin in different diseases of the corium.

BIBLIOGRAPHY

1. BONNIGER, M. Die elastische Spannung der Haut und deren Beziehung zum Oedem, *Ztschr. f. exper. Path. u. Therap.*, **1**:163-83, 1905.
2. BROŽEK, J., and KEYS, A. The evaluation of leanness-fatness in man: norms and interrelationships, *Brit. J. Nutrition*, **5**:194-206, 1951.
3. ———. Body build and body composition, *Science*, **116**:140-42, 1952.
4. CHIEFFI, M. An investigation of the effects of parenteral and topical administration of steroids on the elastic properties of senile skin, *J. Gerontol.*, **5**:17-22, 1950.
5. ———. The effect of topical estrogen application on the elastic properties of the skin of elderly men, *ibid.*, p. 387 (abstr.).
6. DICK, J. C. The tension and resistance to stretching of human skin and other membranes, with results from a series of normal and edematous cases, *J. Physiol.*, **112**:102-13, 1951.
7. DOERKS, G. Zur klinischen Prüfung der Hautdehnung, *Arch. f. Kinderheilk.*, **136**: 1-11, 1949.
8. EVANS, R.; COWDRY, E. V.; and NIELSON, P. E. Ageing of human skin. I. Influence of dermal shrinkage on appearance of epidermis in young and adult fixed tissues, *Anat. Rec.*, **86**:545-60, 1943.
9. GOLDZIEHER, J. W. The direct effect of steroids on senile human skin, *J. Gerontol.*, **4**:104-12, 1949.
10. GOLDZIEHER, J. W., and GOLDZIEHER, M. A. Effects of steroids on the aging skin, *J. Gerontol.*, **5**:385, 1950 (abstr.).
11. GOLDZIEHER, M. A. The effect of estrogens on the senile skin, *J. Gerontol.*, **1**:196-201, 1946.
12. JADASSOHN, J. Eine eigentümliche Furchung, Erweiterung und Verdickung der Haut am Hinterkopfe, *Verhandl. d. 9. Kong. Deutsch. dermat. Gesellsch., Bern*, 1906.
13. JOCHIMS, J. Untersuchungen des mechanischen Verhaltens der Hautgewebe (Cutis + Subcutis) mit einer neuen Methode, *Ztschr. f. Kinderheilk.*, **56**:81-97, 1934.
14. ———. Elastometrie an Kindern bei wechselnder Hautdehnung, *Arch. f. Kinderheilk.*, **135**:228-37, 1948.
15. KIRK, E., and KVORNING, S. A. Quantitative measurements of the elastic properties of the skin and subcutaneous tissue in young adults and old individuals, *J. Gerontol.*, **4**:273-84, 1949.

16. KIRK, J. E. Quantitative measurements of the elastic properties of the skin and subcutaneous tissue in middle-aged individuals, *J. Gerontol.*, **5**:387, 1950 (abstr.).
17. LANGER, K. Zur Anatomie und Physiologie der Haut, *Sitzungsb. d. k. Akad. d. Wissensch.*, pp. 19-179, 1861. Quoted by CHIEFFI (5).
18. MA, C. K., and COWDRY, E. V. Aging of elastic tissue in human skin, *J. Gerontol.*, **5**:203-10, 1950.
19. PINKUS, F. Die normale Anatomie der Haut. *In*: JADASSOHN, *Handb. d. Haut- u. Geschlechtskr.*, **1/1**: 1-378, 1927.
20. ROLLHÄUSER, H. Die Zugfestigkeit der menschlichen Haut, *Gegenbaurs morphol. Jahrb.*, **90**:249-61, 1950.
21. SCHADE, H. Untersuchungen zur Organfunktion des Bindegewebes. I. Die Elastizitätsfunktion des Bindegewebes und die initiale Messung ihrer Störungen, *Ztschr. f. exper. Path. u. Therap.*, **11**:369-99, 1912.
22. ———. Die physikalische Chemie in der inneren Medizin. Dresden and Leipzig: T. Steinkopff, 1921.
23. STUART, H. C., and SOBEL, E. H. The thickness of the skin by age and sex in childhood, *J. Pediat.*, **28**:637-47, 1946.
24. TUI, C.; KUO, N. H.; and SIMUANGCO, S. Protein studies in scleroderma, *J. Invest. Dermat.*, **15**:181-203, 1950.
25. WENZEL, H. G. Untersuchungen über die Dehnbarkeit und Zerreissbarkeit der Haut, *Zentralbl. f. allg. Path. u. path. Anat.*, **85**: 117-18, 1949.
26. WÖHLISCH, E.; DU MESNIL DE ROCHEMONT, R.; and GERSCHLER, H. Untersuchungen über die elastischen Eigenschaften tierischer Gewebe. I. Elastizitätsmodul, Zerreissfestigkeit, Arbeitsvermögen und elastische Vollkommenheit, *Ztschr. f. Biol.*, **85**:325-41, 1927.

CHAPTER 2

Electrical Behavior

I. POLARIZATION CURRENTS	9
II. TONUS POTENTIALS OF P. KELLER	13
III. RESISTANCE OF THE SKIN TO ALTERNATING CURRENT	14
A. Impedance	14
B. Impedance of Normal Skin	15
C. Impedance of the Skin in Dermatoses	16
IV. THE PSYCHOGALVANIC REFLEX	17
V. THE LOCAL GALVANIC SKIN REACTION OF EBBECKE	21

I. POLARIZATION CURRENTS

ELECTROPHYSIOLOGICAL work is done with nonpolarizing electrodes. The skin or other tissues are in contact with a salt or salt solution, which, in turn, contacts the same metal as that contained in the salt as its cation. Mostly the zinc/zinc sulfate, mercury/calomel, and silver/silver chloride systems have been used.¹ If a galvanic current is sent through such a system, the metal is ionized and dissolved into the salt at the anode, and metal is precipitated from the salt solution at the cathode. Thereby a "symmetrical" system is created in which countercurrents (polarization currents) within the electrodes are eliminated. When nonpolarizing electrodes are used, all changes in the current can be attributed to processes taking place in the tissues.

On sending a direct current through the human body by means of nonpolarizing electrodes, the initial intensity of the current drops considerably within a fraction of a millisecond, as if there were a sudden increase in ohmic resistance (Fig. 1). This immediate and sudden change in the intensity

of the current is due to polarization, i.e., to the generation in the body of an electromotive force in a direction opposite to that of the original current. The polarization current is produced in the epidermis exclusively. It fails to appear if the continuity of the epidermis is disrupted. It can be obtained in its full strength if the current is sent through the isolated epidermis.

Because of generation of polarization currents, it seems as if the body would have great resistance: at a given electromotive force, the intensity of the current passing through the skin is extremely low. Since this is not a true ohmic resistance, it has been called a "pseudo-resistance." Its site is in the epidermis. After removal of the epidermis the resistance of the body drops to extremely low values.

Polarization currents are produced in tissues when the mobility of different ions is different in the different media through which they have to move, with or without the influence of the externally impressed current. In the epidermis, polarization is produced in part by such diffusion potentials. The diffusion potentials are much

1. For modern surface electrodes see Kennedy and Travis (41).

greater if the two fluid media containing electrolytes of different composition and concentration themselves have different physical properties, so that the relative mobility is different in the two media. On the surface between two such media there is a tendency to equalize concentrations; but in this attempt either the positively or the negatively charged ions move faster than the ions with opposite charge. This separation cannot go too far because of electrostatic forces which oppose separation of ions with opposite charges. Still it goes far

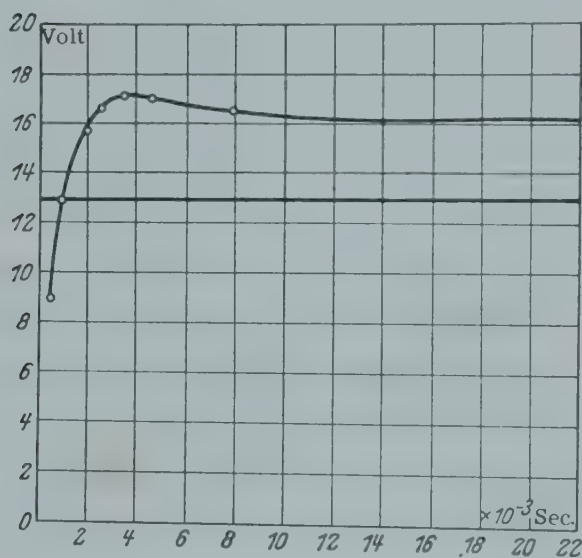


FIG. 1.—Development of polarizing counter-current. From Rein (55). (Reproduced by permission of Springer-Verlag.)

enough to create a difference in charge at two closely situated points.

If a current is sent through the body, diffusion potentials will arise between the electrode fluid and the skin surface, as well as between the skin surface and any point inside the body. There are differences in the potentials at different points on the skin surface. Thus it becomes understandable that, even without applying an external electrical current, just connecting two areas of the skin surface by an electrically conducting circuit, the connection being established by means of nonpolarizing electrodes equal in all respects on both sides, an electrical current will be produced, the intensity of which can be measured with a

microammeter. This happens even under the best possible resting conditions. Thus skin potentials (or resistances induced by the potentials) can be studied either by applying an external current (exosomatic method) or without it (endosomatic method). Potentials measured endosomatically are also called "ground potentials"; and resistances, "static resistance levels" (47).

The potentials measured by the exosomatic method, of course, will depend on the electromotive force of the externally impressed current, on the nature of the electrode fluid, on the pressure exerted by the electrode, and on the size of the surface area of the fluid electrode by which the skin surface is connected with the electrical circuit. The latter factor was studied by I. H. Blank (9) in detail. In the exosomatic arrangement, if the electrode surface area is very large, the density of the electric current (i.e., the electrical intensity per unit area) and the changes due to ionic migration will be very small. Therefore, in studying changes at the site of the electrode, it is sometimes advisable to use one relatively large and one small electrode. In this arrangement little happens at the site of the large or indifferent electrode, but intense effects are produced at the site of the small or active electrode. The active electrode can be connected with either the positive or the negative pole of the galvanic element, these two arrangements being called "anodal" and "cathodal" leads.

The behavior of the polarization on the skin surface is in many respects analogous to that of artificially built porous membranes as studied by Bethe and Toropoff (6, 7) (p. 28). Such membranes selectively absorb either cations or anions in their pores. According to the size and electrical charge of the pores, the membrane may slow down or completely hinder the movement of either cations or anions. They are "ionic sieves" if they are completely impermeable to either cations or anions.

The analogy between ionic sieves and the epidermis was first recognized by Rein (52). He separated the skin from the human body

and found that it behaved like a membrane which had a negative charge (as against water), adsorbing anions and therefore being impermeable to anions. If the isolated skin or epidermis was used as a diaphragm in a vessel filled with water and a direct current was sent through the water, there was a movement of the water from the anode to the cathode. This movement of water through a membrane is called "electro-osmosis." Its direction is toward the cathode if the membrane has a negative charge. Since this was the direction of the current when the skin was the diaphragm, Rein concluded that the skin has a negative charge.

The negative charge of the skin membrane could be diminished by the addition of neutral salts, as demonstrated by the decreasing velocity of the movement of the water through the membrane. The salts were more effective, the higher their concentration and the higher the valency of their cations. They were effective in the order $K < Na < Ca < Al$. Eventually the skin became discharged, when there was a standstill of the endosmotic water movement. Beyond this point the skin became charged positively, and there occurred a reversal of the direction of the water movement. Aluminum ions were remarkably effective in reversing the charge. An alkaline reaction increased the originally negative charge due to adsorption of hydroxyl ions; an acid reaction caused discharge and eventually reversal of charge. Complete discharge occurred between pH 3 and 4.

Most protein membranes have a negative charge in neutral solutions and no charge at the isoelectric point (p. 340). From this point proteins can be charged in either direction because of their amphoteric character (p. 340). The skin surface elements consist of a heterogeneous system of proteins, to which the term "isoelectric point" can hardly be applied. Still it may be worth while mentioning that the pH at which the skin surface *in vivo* is at a minimum charge is identical with the "isoelectric point of the horny layer" (4.1) as established by Marchionini (71) and by P. Keller (38, 39).

Because of the originally negative charge in the superficial skin layers, it is relatively easy to introduce basic dyes (dye cations, e.g., methylene blue) into the skin electrophoretically, whereas acid dyes (dye anions, e.g., eosin) do not penetrate (53) (p. 51). Even without an externally applied electromotive force, dye cations penetrate into the epidermis and the upper part of the follicular epithelium.

Rein (54) studied the potential difference between two skin areas when potassium chloride solutions of different concentration were used as electrode fluids. The mobilities of K and Cl are almost exactly equal, and therefore no potential differences are generated within the electrode fluid. When the concentration of one electrode was kept constant (N KCl) and the concentration on the other was varied from 1/10 N downward, an electrical flow was obtained from the site of the more dilute toward the site of the more concentrated solution. With increasing dilution at the variable electrode, the positivity at this electrode increased. Positivity at the variable electrode develops because the total electrolyte concentration in the skin surface layers is greater than N/10, and therefore the electrolytes of the skin tend to diffuse into the electrode fluid; however, the skin being impermeable to anions, only cations are able to move toward the electrode. This makes the electrode fluid more positive. The potential differences with the concentration differences 1/1,000 N:1/100 N and 1/100 N:1/10 N were found to be greater than the potential differences with 1/10 N:N. This happened because under the influence of relatively high salt concentrations the skin became more and more permeable to anions being discharged or depolarized, and both anions and cations could migrate to equalize differences in concentration.² Table 1 shows the numerical data of the measured potential differences and the reversibility of discharge by salts.

Rein (54) found a remarkable sex differ-

2. For further data on electromotive effects of concentration see Beutner (8).

ence in the polarizing capacity of the skin, weaker in the female than in the male. Among other clear-cut differences, he observed that depolarization of the skin surface, which occurs when a galvanic current is sent through the body continuously, takes place in the skin of women at a more rapid rate than in men.

Rein (55) presented histological and electrophysiological evidence that the high electromotive forces produced at the boundary of fluid and skin are not due to the multiplicity of layers in the epidermis. In other words, high polarization is not due to the fact that several polarizing elements are connected in series. He demonstrated on

TABLE 1 (54)

Constant Electrode	Variable Electrode	Emf (Milli-volts)	Charge of Variable Electrode
N KCl....	0.01 N KCl	20.00	+
N KCl....	0.1 N KCl	4.40	+
N KCl....	N KCl	0.00	0
N KCl....	2 N KCl	7.00	-
N KCl....	0.01 N KCl	19.00	+

lifeless membranes that a single surface (so-called "boundary lamella") can very well be the site of as high potentials as those found in the skin. By changing the concentration of the potassium chloride solutions, Rein found that the changes in the intensity of current were so abrupt and so profound that it was not feasible to attribute this change to subsequent events in subsequent layers.

According to Rein, the boundary at which polarization currents originate cannot be in the horny layer, because the horny layer is a grossly porous membrane in which not only ions but also large molecules move freely. It is easy to demonstrate that any kind of neutral, acid, or basic dye penetrates the horny layer without difficulty. However, such dyes come to a sudden halt at the lower level of the horny layer, and the Malpighian layer remains unstained. Rein concluded that the polarizing mem-

brane is in the stratum lucidum (p. 29), but he probably meant more generally the boundary between noncornified and cornified epidermis (p. 29). I pointed out (70) that this boundary has the characteristics of an electrical double layer, the outside having a strongly acid, and the inside a slightly alkaline, reaction. This situation is analogous to that in Bethe's artificial membrane after an electric current was sent through it.

In spite of the impressive work of Rein, there is still no general agreement concerning this point. Lewis and Zotterman (43), as well as Rosendal (66, 67), for instance, insisted that the site of high resistance to galvanic current is the horny layer, and recently the resistance to alternating current was also interpreted in this way (28).

Recently Pozzo (50) tried a new approach by inserting needle electrodes into different depths of cadaver skin, measuring the distance of the needle points from the surface, and recording the intensity of currents flowing through between two needle points when connected with a galvanic source of 42 volts. No polarization current was obtained from the needle electrodes down to 0.3 mm. depth. At that depth a weak current was obtained. The intensity of the current reached a maximum in some cases at 0.5 mm.; in other cases at 1.2 mm. All one could conclude was that the main site of the polarizing membrane is somewhere below 0.3 mm. If such experiments were controlled histologically, so that the position of the needle point could be established topographically with microscopic accuracy, valuable information could be obtained about the site of the polarizing membrane. The thickness of the horny layer is so variable individually and regionally that, without histological control, it is not possible to decide the question of whether the resistance resides within the dead horny layer, as Lewis and Zotterman and others postulate, or below it. I am inclined to accept the evidence of Rein, mainly because of his findings on dye penetration and because of the electrical double layer characteristic of the

boundary between cornified and noncornified epidermis. However, at the present time it cannot be determined whether, in areas

with a thick horny layer, its lower compact part does or does not participate in forming the polarizing membrane (p. 32).

II. TONUS POTENTIALS OF P. KELLER (38, 39) (ELECTRICAL SKIN POTENTIALS AFTER ELIMINATION OF DIFFUSION-TYPE POTENTIALS)

The data of Rein, reproduced in Table 1, are only expressions of different mobilities of ions inward and outward at the boundary, depending on the difference in concentration of the two electrode fluids. Rein (54) thought that more meaningful values could be obtained if, at the site of one electrode, the resistance at the boundary were completely removed. By superficial scarification of the epidermis, the skin becomes an electrically neutral nonpolarizing medium, with free exchange of ions in all directions. Such scarified areas have become insensitive to changes in concentration of electrode fluids with which they are in contact; and thus diffusion-type potentials have been eliminated. Actually, the potentials measured at such places are conspicuously constant. According to Rein (55), the potential at the injured site can be regarded as a zero point, so that potential differences between this site and sites of intact skin can be regarded as a true measure of the electrical charge of intact skin.

If a scarified spot is connected with a spot of intact skin by nonpolarizing electrodes and by the use of N to 2N KCl as electrode fluid, a current can be observed flowing from the injured area toward the uninjured normal skin (55). In relation to the injured area the normal skin has a negative potential because of better penetration of K^+ than of Cl^- from the electrode fluid into the skin (p. 11), while in the injured area the ions move with equal velocity in both directions.

Keller (38, 39) noticed that it was not necessary to scarify the skin in order to eliminate diffusion potentials and to secure constant potentials. Prolonged soaking of the skin in water or in concentrated salt

solutions had the same result. The most effective method of depolarization was soaking in acids at pH 3.4, which caused the skin immediately to lose its negative charge. In spite of the elimination of the diffusion potential by soaking, such discharged areas were found to be electrically active, inasmuch as when they were connected with an injured area, the soaked areas showed electric negativity as related to the injured spot. Keller found considerable variation in these negative charges in different regions of the body surface. Highest values were obtained on palms, which would suggest that such a potential is due to sweat-gland function. Keller insisted, however, that his tonus potentials are clearly distinct from the potentials of the psychogalvanic reflex (p. 17), the latter being a short-lasting impulse, while Keller's potentials represented a continuous "tonic" state which remained unchanged for 30 minutes or longer. Still the potentials obviously were connected with nervous activity. Atropinization caused disappearance of the charge on palms and flexor surfaces of forearms and a slight decrease of charge on the forehead. The charge of the skin on chest, upper arms, and extensor aspects of forearms, however, did not change after atropinization. Potentials decreased by atropinization could be further decreased by procainization. Therefore, it was thought that if the potentials measured in this way are a function of nervous activity, they are not merely due to sudomotor impulses but are the expression of a more general nerve tonicity.

On the basis of Keller's work, Woeber and Hogrebe (79, 78) depolarized skin areas by soaking with concentrated potassium chloride solution. Subsequently, they con-

nected the soaked area with an area of untreated normal skin. Saturated potassium chloride solution was used on the soaked spot as the electrode fluid, and a weaker salt solution on the normal skin. In this way Woeber obtained negative charges on the normal skin ranging from -10 to -40 mv, the range being due to individual and regional differences as well as to day-by-day variations. They also measured potentials on normal mucous membranes and found lower values there, namely, -2 to -20 mv.

If one lead was taken from intact skin with saturated KCl solution and the other lead from a naturally diseased lesion with N/10 KCl solution, positive potentials up to 70 mv were obtained in the diseased lesions, regardless of whether the epidermis was grossly broken or not. It was found that the positive potentials were (1) higher in acute than in chronic inflammatory processes; (2) higher on the surface of rapidly growing than on slowly growing lesions; (3) low in torpid ulcerations but rapidly increasing when healing set in. In healing granulating ulcers the originally positive potentials dropped to zero when epithelization set in; and after the healing process was completed,

the physiological negative potential was restored.

It was concluded that if the epidermis is intact, it always has a negative charge relative to an area which is depolarized by saturated potassium chloride solution. As soon as the epidermis suffers some damage, this negative charge disappears, and the charge of the damaged skin becomes identical with that of depolarized skin. The pathological process progressing under the damaged epidermis produces a positive charge. The more proliferative this process, the higher is the positivity.

It should be pointed out that the negative charge of normal skin as contrasted with the positive charge of damaged skin is in contrast with the behavior of muscle. If the intact surface of a muscle is connected with an injured spot of the same muscle, a current will flow from the relatively positive intact surface to the relatively negative damaged spot.

A conspicuously large decrease of galvanic resistance was found by Battaglini (5) in lupus erythematosus lesions; the decrease was less in "eczema" and very much less in lesions of psoriasis.

III. RESISTANCE OF THE SKIN TO ALTERNATING CURRENT

A. IMPEDANCE (36)

The total opposition exerted by a circuit to the flow of an alternating current is called the "impedance" of the circuit. This opposition is different in nature from simple ohmic resistance to direct currents.

Whenever a current flows through a coil, a counter electromotive force is induced in the coil. This induced voltage tends to oppose any change in the current flow; and, therefore, in the case of alternating currents a counter electromotive force is induced continuously. Consequently, the opposition of the coil to the flow of an alternating current will be greater than that to the flow of a direct current. The impedance exerted by a coil by virtue of inductance is called its "inductive reactance." It is expressed in ohms. Its value increases proportionally to

the frequency of the alternation of the current.

If the circuit has condenser properties, in other words, if it contains materials with high conductance separated by materials with high dielectric properties, an alternating current will alternately charge and discharge the condenser, which, in turn, will oppose these changes. This opposition is called "capacitance reactance." Its value is expressed in ohms. It is inversely proportional to the capacitance of the condenser and to the frequency of the current.

Both reactances cause a displacement of the periodicity of the alternating current. The shift of the phase is measured geometrically by an angle called the "phase angle" and is expressed either directly in degrees or as the tangent of the angle.

B. IMPEDANCE OF NORMAL SKIN

Early measurements of the impedance of human skin (30, 31) revealed that the skin exerts a capacitance reactance to the flow of an alternating current in the audiofrequency range (periods of from 100 to 3,200 per second). The reactance tended to increase with decreasing frequency. The early data, however, did not reflect clearly the impedance of the skin proper, because the values obtained included the impedance of deeper tissues. Moreover, the data of different authors were not in good agreement (12).

Horton and van Ravenswaay (37) worked out a four-electrode method whereby it became possible to measure separately the impedance of the skin ("surface sheath") and that of deeper tissues ("inner impedance"). Barnett (1) further developed and perfected the multielectrode principle. His data actually represent solely the impedance of the skin. Barnett found that the high resistance of the "external sheath" resides in the epidermis. With his special annular electrodes, 6 sq. cm. in area, this impedance was of the order of 100 ohms in normal subjects in the winter months, gradually increased in spring to a maximum of 140–250 ohms in summer, and declined again, starting in October (4). The same seasonal variations were found to be present also in institutionalized psychotic patients, who were hardly exposed to solar radiation, variations in temperature, and other atmospheric changes. Barnett thought the observed seasonal variations could be best explained by variations in the thickness of the epidermis (see below).

Barnett (2) found the phase angle of the skin at 15,300 cycles between 64° and 78° , with a mean value of 71.3° and a standard deviation of 2.85° , a fairly narrow range. The mean value for males was 71.6° , for females 70.9° . In the range of frequencies between 200 and 2,000 cycles the phase angle remained constant. Such behavior occurs only in simple structures, and it was rather surprising to find it in the multilayered and heterogeneous epithelium. Barnett believed that this might be due to a "close packing"

of the cells. Another explanation may be that the site of capacitance reactance is a single surface at the border of noncornified epithelium, as is the site of the galvanic pseudo-resistance, according to Rein (p. 12). Barnett's results were confirmed by Geets and Colle (26).

Gerstner (27) used a different method to separate the impedance of the skin from that of deeper structures. He achieved the separation by the use of carbon powder-saline electrodes. With this method he found a capacitance reactance in the skin which changed geometrically with the frequency of the current, the factor being 2 with octaves in cycles. He stated that with his method that part of the impedance was measured which was due to ion stasis in the presence of ion-impermeable membranes. In parallel with the lower resistance to galvanic currents in females (p. 12), Gerstner found in low frequencies (up to 1,600 per second) a lower capacitance reactance in women than in men. He demonstrated that this reactance increased with age up to about forty-five and became less again between sixty and seventy. In low-frequency ranges there were definite regional differences in impedance, such as, for instance, ball of thumb > forearm > abdomen > forehead, an order reminiscent of regional differences in "ground potentials" (p. 13). However, he also found that if a person had unusually high or unusually low impedance on one part of the body surface, the values on other parts were correspondingly high or low. Therefore, he thought that the cutaneous impedance could be regarded as a general characteristic trait of the individual.

On the buccal mucous membrane Gerstner found lower impedance values (phase angle below 45°) than on the skin. After death the impedance of the skin did not change essentially. In isolated pieces of cadaver skin the values were comparable to values obtained in vivo. Even the sex differences could be demonstrated in cadaver skin.

In a subsequent paper Gerstner and

Gerbstadt (28) standardized the method. They found that one can obtain results with good reproducibility if the electric density at the electrodes is below 0.015 M.A./sq cm, if the pressure exerted by the electrode is less than 100 gm/sq cm and if the concentration of the sodium chloride solution in the electrode is 1 per cent or less.

Furthermore, they attempted to study the site of the capacitance reactance in the skin by measuring it before and after burning, scarification, and sandpapering. They found that the separated tops of their burn blisters had the same reactance as that of the total skin, while the reactance of the denuded skin, after removal of the blister top, dropped to almost zero, the skin behaving almost like a nonpolarizable ohmic resistance. The reactance could be abolished also by superficial scarification or by sandpapering.

The authors concluded that the site of reactance must be localized in the horny layer, as was assumed previously by others (29, 22). They attempted to substantiate this conclusion by histological examination. They definitely could demonstrate that the thicker the horny layer was, the higher the impedance of the skin.

The difficulty in accepting this conclusion is the same as in the case of polarization in response to direct current. The authors did not determine histologically whether their blister top actually consisted of only horny layer (although they called it that) and did not control histologically the depth of damage caused by scarification and sandpapering. As a matter of fact, studying their data, I found that weak and fine damage to the skin in vivo and in cadaver skin did not substantially decrease the impedance. The question is still debatable as to whether the capacitance reactance, like the pseudo-resistance to galvanic current, has its site in the horny layer proper or between the cornified and noncornified layers (pp. 12 and 29).

It has been known for a long time that the electrical behavior of the skin is abnormal in diseases of the thyroid gland (11).

A high basal metabolic rate is connected with low impedance, and the opposite is true for a low basal metabolic rate.

Barnett (3) demonstrated that the lowered impedance in thyrotoxicosis does not involve any change in the phase angle; and he interpreted this type of change as being due to a change in the thickness of the condenser without change in the dielectric material. In other words, in thyrotoxicosis the epidermis is thinner, in hypothyroidism thicker, than normal, but there are no qualitative changes. Barnett found in mentally diseased persons subnormal values of impedance such as those which occur in thyrotoxicosis, and he speculated that the thinning of the epidermis in these cases might be due to a deficiency in pituitary hormones.

Gerstner (27) found high impedance in patients suffering from internal malignancies (due to thickening of the epidermis?).

C. IMPEDANCE OF THE SKIN IN DERMATOSES

L. Gougerot (34) was the first to compare impedance of normal and of pathologically altered skin. He used an alternating current with 4,000 cycles per second, saline-soaked filter-paper electrodes, 8.5 sq. cm., and took leads from the extensor and flexor aspects of forearms and lateral surfaces of the thighs. On normal skin the tangent of the phase angle was always greater than 0.88, and the impedance greater than 150 ohms. In forty-two measurements average values were: phase-angle tangent 1.45, impedance 300 ohms. In forty-one measurements on twenty-three patients with active lesions of "eczema" or "eczematized microbial dermo-epidermite," all values were pathologically low. The phase-angle tangent was always less than 0.90, and the impedance was always below 190 ohms. The difference was actually more pronounced than these data would indicate, since in only four out of forty-one measurements did the phase-angle tangent overlap normal values, and only in these four cases was the impedance higher than 150 ohms. The average patient values

were: phase-angle tangent 0.50 and impedance 90 ohms.

In seven cases Gougerot studied uninvolved and involved skin in the same person, and in all of them the tangent of the phase angle was twice to four times greater in normal than in diseased skin.

Registering the electrical behavior of the "eczematous" skin lesions during the healing process, Gougerot was able to distinguish two different types: there was either a gradual return to normal values, or, in spite of obvious clinical improvement up to almost complete cure, the low values persisted. In eczematous lesions he noted a most unusual constancy of the phase angle with changing frequency of the alternating current in the range of 200–750 frequencies. This abnormal behavior did not revert to normal when the skin lesion healed. In some cases the normal-appearing skin surrounding the eczematous patches also yielded pathologically flat phase-angle-tangent/frequency curves.

In contrast to eczematous skin lesions, the healed skin at the site of previous lupus vulgaris lesions showed in four out of five cases phase-angle curves and resistance values far above normal. In psoriasis the involved skin yielded normal values when the lesions were dry, but low values over exudative lesions. However, the uninvolved skin of psoriatic patients yielded throughout abnormally high values—an entirely new and unexpected feature of the constitutionally different nature of the skin in psoriasis.

The short case histories of Gougerot suggest that the majority of his "eczema" cases corresponded with what is known as atopic dermatitis or exudative neurodermatitis in American terminology. Gougerot emphasized the fact that with clinical improvement there is no corresponding reversal in electrical behavior in these cases.

The abnormalities in active exudative lesions could be expected. They could be satisfactorily explained by spongiosis, which implies that there are "actual holes" in the epidermis, a state which H. Gougerot called a "state of porosity," which obviously destroys epidermal resistance altogether. However, it was not to be expected that abnormalities would be found in healed lesions with normal appearance or in the areas surrounding active lesions. In the opinion of Gougerot, low values in apparently normal skin have a pathogenetic and prognostic significance. He believes that in "eczema" the increased permeability of the epidermis, as manifested in low phase angle and low impedance values, is an element of the "eczematous terrain" or, in other words, a constitutional trait of the skin in this disease.

The significance of these findings for investigational work in the field of psoriasis and atopic dermatitis is obvious. It certainly would be desirable to have Gougerot's data duplicated on a large number of patients, with particular emphasis on the uninvolved skin in psoriasis and in spongiotic dermatides. Possibly in these two groups of patients there is a constitutionally abnormal skin, the permeability of the epidermis being abnormally low in psoriasis and abnormally high in exudative neurodermatitis. In the latter disease the "fragility" of the epidermis—an unusually easy breakdown in response to mild injury—has been noted by clinicians many times.

One should remember that, according to Gerstner (27), the level of skin impedance is a characteristic individual trait. It is possible that exudative neurodermatitis patients are recruited among individuals with genetically subnormal impedance, while psoriasis develops in individuals with genetically high impedance.

IV. THE PSYCHOGALVANIC REFLEX

The term "psychogalvanic reflex" is objectionable because the same reflex impulse can be elicited after elimination of the cerebral cortex (60, 73). The name "galvanic

skin response" or "galvanic skin reaction" has been suggested instead. However, there are two galvanic skin responses, one depending on sweat-gland activity (the psycho-

galvanic reflex) and one independent of glandular function (the local galvanic reaction of Ebbecke) (p. 21). Therefore, in this section, for clarity's sake, the term "psychogalvanic reflex" is retained.

In 1890 Tarchanoff (74) reported on the following observation: on connecting two skin areas in man by means of nonpolarizing electrodes, interposing a sensitive galvanometer, and stimulating the subject by touch, light, or auditory stimuli or by eliciting mental effort or emotions, the galvanometer registered a current. The current was particularly marked if one of the electrodes was attached to the palm, sole, or axilla. Its direction was always such that the area with greater sweat-gland supply became electro-negative.

The arrangement of Tarchanoff was modified by Müller and by Veraguth (75) in 1904 by interposing a galvanic element into the circuit. When a direct current flowed through the body, stimulation of sense organs or psychic stimuli caused a great increase in the intensity of the exosomatic current. Veraguth coined the name "psychogalvanic reflex."³

It was shown first by Gildemeister (29) that in this reaction the true ohmic resistance does not change but that there is an increase in electromotive force because of a decrease in polarization currents. It is a general biological rule that the resting cells have a high polarizing capacity and that, on stimulation, this decreases because of increased permeability of their membranes.

It was recognized early that sweat-gland activity is essential for this reaction. The supposition that smooth-muscle contraction plays a role in it has been abandoned, and participation of vasomotor impulses has never been satisfactorily evidenced (47). The reaction is abolished by peripheral nerve section or by sympathetic ganglionectomy (62), an indication that the efferent part of the reflex is carried in sympathetic fibers. This alone, of course, would not exclude sympathetic vasoconstrictor impulses.

3. For modern methodology, apparatus, etc., see references 35, 77, 72.

However, Darrow (13, 14), who simultaneously recorded galvanic skin response, plethysmographic changes, blood pressure, and accumulation of moisture on the skin surface, showed conclusively that changes in volume attributable to vasomotor responses were not related in any way to the occurrence or amplitude of the galvanic skin response.⁴ Moreover, the great majority of investigators found that atropinization abolishes the reaction, a finding which at least strongly suggests the primary role of sweat glands. It must be kept in mind that atropine has some vasomotor effects. The static resistance of the skin is influenced by atropine (p. 13), and the psychogalvanic skin response is greater, the higher the static resistance. However, no reservation needs to be made in the evaluation of Wagner's observation (76). He found that the galvanic skin response could not be elicited in persons with congenital absence of eccrine sweat glands.

Certainly, the fact that the response to psychic stimuli can be elicited consistently only from palms and soles and that these areas give reactions of greatest amplitude again points to the decisive role of sweat glands, because the sweat nerves of these areas are known to be most responsive to psychic stimulation.

In chapter 6 (p. 177) a detailed account is given of observations indicating that sweat glands of palms and soles are more conditioned to respond to sensory and mental stimuli than to thermal stimulation and, conversely, that sweat glands of head, trunk, and extremities (except palms and soles) are primarily conditioned to respond to thermal stimuli, axillary eccrine glands taking an intermediate position.⁵ While these differences are indeed easily demon-

4. Recently presented data indicate that the resistance response can be abolished by complete exsanguination. It was concluded that the response has a vascular component (32).

5. That sweating in the axilla in response to mental stimuli is not a function of apocrine glands is concluded from the observation that there is no emotional sweating in the perimamillary areas.

strable (42, 58), there is no evidence whatsoever that palmar and plantar sweat glands respond *only* to sensory and mental stimuli and other sweat glands *only* to heat stimuli. Following exercise, for instance, the electrical resistance of the palms decreases considerably (9), and, on the other hand, startling stimuli easily elicit "cold sweat" all over the body surface (p. 174).

The differences in responses of palms and soles as compared with those of other areas were studied in great detail by Darrow (20) and by Richter (58). The latter specifically compared reactions obtained from palms and dorsa of hands, varying heat and emotional stimuli and examining responses in persons awake and asleep. He found that, whereas the palms always responded to mental stimulation with increased sweat-gland activity, the responses from the dorsum of the hand were not regular and not clearly due to sweat-gland activity. Still, the dorsum of the hand responded to atropine and to a warm bath in just the way one would predict if sweat glands were the basis of the response. Yet it appeared that responses on the dorsa of hands have a different mechanism or, at least, that, in addition to sweat-gland activity, there must be some other factor. Richter has found that after denervation of the upper extremity in monkeys the palm acquires a high resistance and does not give any galvanic response, while the dorsum of the hand shows little postoperative change. Richter (56, 57, 59, 61) also observed that the palmar resistance rises in sleep, while in other places the resistance decreases, and that the palmar sweat glands do not take an appreciable part in thermoregulatory sweating.⁶

From a review of the vast literature of the

6. Palmar resistance has been used as a criterion of depth of sleep. The resistance reaches its peak at the end of the third quarter of the night's sleep. At the same time the heart rate is lowest. If acoustic stimuli, blood pressure, or minimum body temperature are used as criteria of depth of sleep, the maximum depth is found to occur earlier during the night's sleep (N. Kleitman, *Sleep and wakefulness* [Chicago: University of Chicago Press, 1939], pp. 31-37).

psychogalvanic reflex one can conclude that on palms and soles the functional state of sweat glands contributes most to the total galvanic pseudo-resistance and, therefore, that the changes in this functional state are the most prevalent factors. In other parts the resistance deriving from epidermal polarization is predominant, and the differences which may arise from the presence or absence of glandular action currents is less conspicuous (p. 21). One may speculate that the thickness and tighter texture of the horny layer on palms and soles may have something to do with the relative insignificance of epidermal resistance. The current through the electrode fluid possibly does not reach the polarizing membrane of the epidermis so easily on palms and soles as it does elsewhere, but reaches the sweat glands easily through the ducts. The relatively low static resistance of palms (38, 64) can be interpreted in this way. The axillae, the central parts of the face, and the cubital fossae also have relatively low resistance (63, 64); and Richter believes that this is due to relatively rich sweat-gland supply. Richter (58) suggested a long time ago that in nonpalmar and nonplantar regions the skin resistance depends entirely on the condition of epidermal cells and responds mainly to local stimuli (p. 21).

The decrease in resistance in the psychogalvanic reflex does not depend on secretion of fluid by the sweat glands but is caused primarily by an increase in the membrane permeability of the secretory cells, whereby polarization currents decrease. The filling of the ducts and the imbibition of sweat by the horny layer, causing a well-conducting electrolyte solution, certainly decreases skin resistance. But it was shown in ingenious experiments by Darrow (17, 18) that the psychogalvanic reflex does not depend on the actual moisture found in or on the skin. Although there is a direct relationship between resistance change and amount of perspiration (19, 25), this relation is not consistent. At high resistance levels, responses of large amplitude are obtained with minimal perspiration, and at low resistance

levels responses of small amplitude occur with considerable perspiration. The reaction can be elicited on profusely sweating moist areas (33), indicating that even a hyperactive gland might be further stimulated. Richter (62) stated that the "fully activated" sweat glands of some persons produce very little or no sweat, while those of others produce large amounts even when only partially activated. Finally, Darrow (16) demonstrated that electrical changes precede the secretion of sweat by about 1 second. Obviously, the psychogalvanic reflex is analogous to other action currents (31).

Darrow (15) developed the ingenious idea that the sweating reaction of palms represents a preparatory and facilitative reflex to assist in grasping responses and to increase tactile sensitivity in the face of a threatening situation. He differentiated startling sensory stimulations from stimuli with disturbing ideational content and found that, for the first type, the galvanic response and, for the second type, blood-pressure responses, are more characteristic.

Mammals, except man and some apes, have eccrine glands exclusively on the palmo-plantar surfaces (p. 180). In the light of Darrow's theory, it seems that eccrine glands in their phylogenetic first appearance had nothing to do with thermoregulatory functions but were created for an entirely different purpose.

As mentioned initially, the term "psychogalvanic reflex" has become obsolete, since it can be elicited after connections with the cortex have been severed. Richter (60) was able to obtain the reaction in all extremities after mid-thoracic spinal transection in cats. After removal of the cortical center of sweating in the premotor area (p. 173), Schwartz (73) could not elicit the reflex by "psychic" stimuli but obtained it in full strength on nociceptive stimulation. Obviously, the situation is as in long reflex sweating in general (p. 173): sweating has a cortical representation, and therefore afferent sensory

impulses may act by reaching this center and then being switched from there to autonomic sweating pathways; however, the same sensory impulses may also be relayed to sympathetic pathways in lower centers, and the end-result will be the same.

For the dermatologist the literature on the psychogalvanic reflex is most instructive because it illustrates how sensitive certain nerve pathways of sweat secretion are to minute psychic stimuli.⁷ This fact may help the understanding not only of purely functional hyperidrosis but also of "emotional" influences on somatic skin diseases which are aggravated by increased sweat secretion. Recently much attention has been paid to this type of aggravation, and it was pointed out that the harmful effect on inflammatory skin diseases is particularly pronounced when the sweat cannot evaporate freely because of closure of the sweat-gland pores (chap. 11, pp. 270 ff.).

It should be emphasized that psychic stimuli which elicit the psychogalvanic reflex do not necessarily have any "emotional" connotation in the colloquial sense of the word. Emotionally indifferent mental effort, such as an attempt to solve simple arithmetical tasks (42), suffices to elicit increased palmar sweating. Duffy and Lacey (23) enumerate the following mental states as being equally effective: increased attention, startle or tension, sudden checks in comprehension and solution of problems, heightened consciousness, conation, states of anxiety, anticipation, alertness, and apprehension. Certainly, no specificity of the psychic stimulus can be recognized (47). Furthermore, it should be stated that the effect on skin diseases is the same whether the increased sweat secretion is elicited psychically, thermally, pharmacologically, or otherwise. The counteracting effect of parasympatholytic drugs is also the same in either case.

7. How sensitive the reaction is, is best illustrated by its use in hearing tests (10).

V. THE LOCAL GALVANIC SKIN REACTION OF EBBECKE (24)

On local stimulation of the skin by moderately intense mechanical, thermal, and electrical stimulation, a marked and reversible decrease of the pseudo-resistance of the skin is observed. This reaction lasts about 40 seconds. It is independent of nervous impulses and of glandular activity and thus entirely different in its nature from the psychogalvanic reflex. Also it is independent of changes in blood flow. The electrical change is strictly limited to the area of stimulation. The rapid subsidence of the reaction indicates that it is not caused by some gross injury the healing of which would require some time.

Ebbecke interpreted the phenomenon as a vital reaction of epithelial cells connected with depolarization of their membranes. He found that the reaction was abolished by "paralyzing" the epidermis with chloroform, obviously because the treatment with chloroform, by damaging cell membranes (p. 49), in itself causes complete depolarization.

In contrast to Ebbecke (24), Lewis and Zotterman (43) interpreted the phenomenon as being due to chafing of the horny layer. Rein (55) stated, however, that if actually only the horny layer is removed, the reaction can still be elicited. He emphasized that the horny layer, as a grossly porous membrane, cannot play any role in electrical reactions which are based on depolarization, and therefore the site of the reaction must be within the living epidermis.

Like the psychogalvanic reaction, this local reaction, too, comes about by development of a current within the tissues. But the different nature of the latter was proved beyond doubt by the following observations: (1) in the local reaction the stimulated area becomes electropositive in relation to the nonstimulated environment, whereas in the psychogalvanic reflex the sweat glands acquire a negative charge; (2) the countercurrent in the tissue following local stimulation can be well demonstrated after atropiniza-

tion; (3) the psychogalvanic reflex can be elicited as a superimposed reaction from the locally stimulated area.

Rein (55) showed that the reversible positive charge can be elicited by extremely mild stimulation, such as touch or mild pressure. Figure 2 illustrates his experiment, in which he applied 20 gm/sq mm pressure on the skin and measured the electromotive force of the countercurrent. On repetition

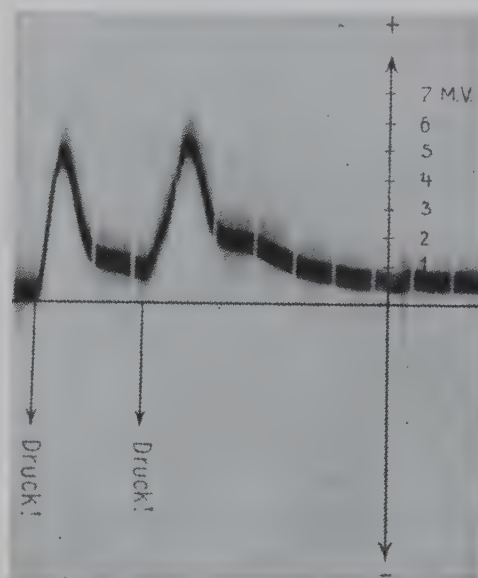


FIG. 2.—Ebbecke's local galvanic reaction on forearm skin. Pressure stimulus ("Druck!") 20 gm/mm both times. N KCl electrode. Neutral electrode on scarified skin. Time signals = 10 sec. From Rein (55). (Reproduced by permission of Springer-Verlag.)

of the same stimulus, the same response was again elicited. Therefore, Rein concluded that physical factors, such as the squeezing-out of fluid through the pores, cannot play any role in the reaction. Interplay of such factors would not be compatible with the perfectly reversible and reproducible course of the reaction. For contrast purposes, Rein's illustration of a psychogalvanic response is also reproduced here (Fig. 3). Mild heat also elicits the local galvanic response (55).

Peculiarly, stimulation of human skin

with ultraviolet rays does not provoke this reaction. Prior to the development of solar erythema, there is some increase in the pseudo-resistance. At the height of the erythema the resistance is lowered (51). The low pseudo-resistance at the height of inflammation is obviously due to gross epidermal damage, with more or less complete depolarization. After the erythema has subsided, the pseudo-resistance reaches unusually high values and stays at such high levels for about 20 days (40). At that time nothing can be seen on the skin except

inflammatory increase in electrical resistance coincides with a period of increased resistance to chemical stimuli and to repeated ultraviolet irradiations.

Yet this problem cannot be regarded as settled. Ultraviolet irradiation is followed by measurable thickening of the horny layer (48), and there is reason to believe that this thickened horny layer is less "porous" than the normal stratum corneum, because of the "superkeratinization" phenomenon (p. 378). While satisfactory evidence was presented that the *normal* horny layer does not inter-

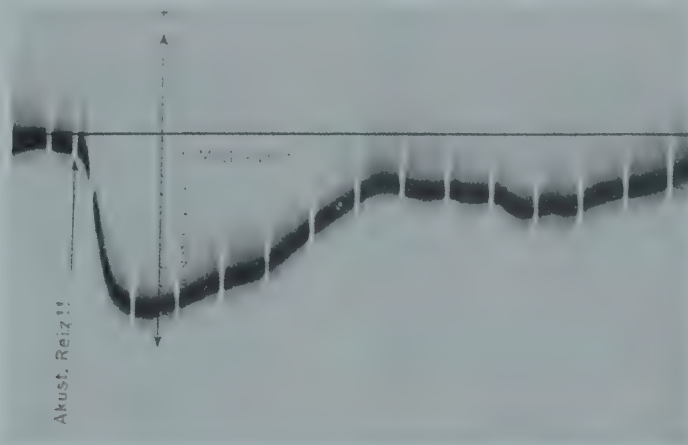


FIG. 3.—Psychogalvanic reflex as a variation of ground potential. Taken from the radial surface of right forearm with N KCl electrode, free of pressure, according to P. Keller. Neutral electrode on scarified skin. Auditory stimulation ("Akust. Reiz!!"). Time signals = 10 sec. From Rein (55). (Reproduced by permission of Springer-Verlag.)

minimal faint pigmentation. This postirradiation, posterythema effect was interpreted by Rost and Keller (69) as an expression of decreased cell membrane permeability (or membrane "densification") and subsequent increased polarization in the epidermis. They pointed out that a post-

fere with electrical phenomena, evidence is lacking concerning a horny layer which is not only thicker but also denser than normal. Such a horny layer may be responsible for increased resistance to chemical, thermal, and actinic stimuli, as well as for an altered electrical behavior.

BIBLIOGRAPHY

1. BARNETT, A. Basic factors involved in proposed electrical methods for measuring thyroid function; phase angle and impedance of skin, *West. J. Surg.*, **45**:540-54, 1937.
2. ———. The phase angle of normal human skin, *J. Physiol.*, **93**:349-66, 1938.
3. ———. Skin impedance findings in mental disease, *Proc. Soc. Exper. Biol. & Med.*, **41**:697-99, 1939.
4. ———. Seasonal variations in the epidermal impedance of human skin, *Am. J. Physiol.*, **129**:P306, 1940.
5. BATTAGLINI, S. Contributo allo studio della reattività, cutanea con speciale riferimento al metodo elettrometrico, *Gior. ital. di dermat. e sif.*, **80**:79-113, 1939.
6. BETHE, A., and TOROPOFF, T. Über elektrolitische Vorgänge an Diaphragmen. I. Die

- Neutralitätsstörung, Ztschr. f. phys. Chem., **88**:686-742, 1914.
7. ———. Über elektrolytische Vorgänge an Diaphragmen. II. Die Abhängigkeit der Grösse und Richtung der Konzentrationsänderungen und der Wasserbewegung von der H-Ionenkonzentration, *ibid.*, **89**:597-637, 1915.
 8. BEUTNER, R. Bioelectricity. In: GLASSER, O., Medical physics, pp. 35-88. Chicago: Year Book Publishers, 1944.
 9. BLANK, I. H., and FINESINGER, J. E. Electrical resistance of the skin: effect of size of electrodes, exercise and cutaneous hydration, Arch. Neurol. & Psychiat., **56**:544-57, 1946.
 10. BORDLEY, J. E.; HARDY, W. G.; and RICHTER, C. P. Audiometry with the use of galvanic skin-resistance response, Bull. Johns Hopkins Hosp., **82**:569, 1948.
 11. BORUTTAU, H., and MANN, L. Handb. d. ges. medizinischen Anwendungen der Elektrizität. Leipzig: W. Klinkhart, 1909. Quoted by REIN (55).
 12. COLE, K. S. Electric conductance of biological systems, Cold Spring Harbor Symp. Quant. Biol., **1**:107-16, 1933.
 13. DARROW, C. W. Sensory, secretory and electrical changes in the skin following bodily excitement, J. Exper. Psychol., **10**:197-226, 1927.
 14. ———. The galvanic skin reflex and finger volume changes, Am. J. Physiol., **88**:219-29, 1929.
 15. ———. Differences in the physiological reactions to sensory and ideational stimuli, Psychol. Bull., **26**:185-201, 1929.
 16. ———. The relation of the galvanic skin reflex recovery curve to reactivity, resistance level, and perspiration, J. Gen. Psychol., **7**:261-73, 1932.
 17. ———. Quantitative records of cutaneous secretory reactions, *ibid.*, **11**:445-53, 1934.
 18. ———. The significance of the galvanic skin reflex in the light of its relation to quantitative measurements of perspiration, Psychol. Bull., **31**:697-98, 1934.
 19. ———. The galvanic skin reflex (sweating) and blood pressure as preparatory and facilitative functions, *ibid.*, **33**:73-94, 1936.
 20. DARROW, C. W., and FREEMAN, G. L. Palmar skin-resistance changes contrasted with non-palmar changes and rate of insensible weight loss, J. Exper. Psychol., **7**:739-48, 1934.
 21. DIEHL, F. Studien zur Permeabilität der menschlichen Haut unter verschiedenen Bedingungen und bei Erkrankungen der Schilddrüse, Ztschr. f. d. ges. exper. Med., **100**:145-91, 1937.
 22. ———. Zur Histologie der Haut bei Morbus Basedowii und Myxödem, Klin. Wchnschr., **22**:386-88, 1943.
 23. DUFFY, E., and LACEY, O. L. Adaptation in energy mobilization: changes in general level of palmar skin conductance, J. Exper. Psychol., **36**:437-52, 1946.
 24. EBBECKE, U. Die lokale galvanische Reaktion der Haut. Über die Beziehung zwischen lokaler Reizung und elektrischer Leitfähigkeit, Arch. f. d. ges. Physiol., **190**:230-69, 1921.
 25. FREEMAN, G. L., and DARROW, C. W. Insensible perspiration and the galvanic skin reflex, Am. J. Physiol., **111**:55-63, 1935.
 26. GEETS, W., and COLLE, J. Mesure de déphasage et d'impédance électrique de la peau humaine, Compt. rend. Soc. de biol., **140**:701-4, 1946.
 27. GERSTNER, H. Statistische Untersuchungen über den Wechselstromwiderstand der menschlichen Haut im Tonfrequenzgebiet, Arch. f. d. ges. Physiol., **250**:125-40, 1948.
 28. GERSTNER, H., and GERBSTADT, H. Der Wechselstromwiderstand der menschlichen Haut, Arch. f. d. ges. Physiol., **252**:111-222, 1949.
 29. GILDEMEISTER, M. Der sogenannte psychogalvanische Reflex und seine physikalisch-chemische Deutung, Arch. f. d. ges. Physiol., **162**:489-506, 1915.
 30. ———. Die passiv-elektrischen Erscheinungen in Tier- und Pflanzenreich. In: BETHE, Handb. d. norm. u. path. Physiol., **8/2**:657-702, 1928.
 31. ———. Die Elektrizitätserzeugung der Haut und der Drüsen. In: BETHE, Handb. d. norm. u. path. Physiol., **8/2**:766-77, 1928.
 32. GOADBY, K. W., and GOADBY, H. K. The nervous pathway of the psychogalvanic reflex, J. Physiol., **109**:177-84, 1949.
 33. GOLLA, F. L. The objective study of neurosis, Lancet, **2**:215-21, 1921.
 34. GOUGEROT, L. Recherches sur l'impédance cutanée en courant alternatif de basse fréquence au cours de différent dermatoses, Ann. de dermat. et syph. (ser. 8), **7**:101-11, 1947.
 35. HAGGARD, E. A., and GERBRANDS, R. An

- apparatus for the measurement of continuous changes in palmar skin resistance, *J. Exper. Psychol.*, **37**:92-98, 1947.
36. HOLMQUEST, H. J. Physical therapy. In: GLASSER, O., *Medical physics*, pp. 1074-93. Chicago: Year Book Publishers, 1944.
 37. HORTON, J. W., and VAN RAVENSWAAY, A. C. Electrical impedance of the human body, *J. Franklin Inst.*, **220**:557-72, 1935.
 38. KELLER, P. Die biologischen Grundlagen für die elektrischen Potentiale der Haut, *Arch. f. Dermat. u. Syph.*, **160**:136-39, 1930.
 39. ———. Grund- und Tonuspotentiale der menschlichen Haut, *ibid.*, **162**:582-610, 1930-31.
 40. KELLER, P., and REIN, H. Polarisationsmessungen an der Haut nach Röntgen- und Ultraviolettlichtbestrahlungen, *Arch. f. Dermat. u. Syph.*, **155**:67-70, 1928.
 41. KENNEDY, J. L., and TRAVIS, R. C. Surface electrodes for recording bioelectric potentials, *Science*, **108**:103, 1948.
 42. KUNO, Y. The physiology of human perspiration. London: J. & A. Churchill, Ltd., 1934.
 43. LEWIS, T., and ZOTTERMAN, Y. Vascular reactions of the skin to injury. VIII. The resistance of the human skin to constant currents, in relation to injury and vascular responses, *J. Physiol.*, **62**:280-88, 1926-27.
 44. LUEG, W. Haut und Elektrokardiogram, *Arch. f. d. ges. Physiol.*, **212**:649-50, 1926.
 45. ———. Elektrochemische Untersuchungen der menschlichen Haut, *Ztschr. f. klin. Med.*, **106**:21-27, 1927.
 46. LUEG, W., and GRASSHEIM, K. Vergleichende Untersuchungen über Grundumsatz und Polarisationskapazität der Haut in Bezug auf die Funktion der Schilddrüse, *Klin. Wchnschr.*, **7**:647-48, 1928.
 47. MCCLEARY, R. A. The nature of the galvanic skin response, *Psychol. Bull.*, **47**:97-117, 1950.
 48. MIESCHER, G. Das Problem des Lichtschutzes und der Lichtgewöhnung, *Strahlentherapie*, **35**:403-43, 1930.
 49. PESCHKOWSKY, P. Über Aenderungen der Polarisationskapazität der Haut unter dem Einfluss einiger Reizmittel, *Ztschr. f. klin. Med.*, **111**:179-90, 1929.
 50. Pozzo, G. Le lesioni cutanee da elettricità. II. Prove sperimentale delle resistenze elettriche dei vari strati della cute, *Gior. ital. di dermat. e sif.*, **90**:382-90, 1949.
 51. REGELSBERGER, H. Über den Galvanismus der menschlichen Haut. I. Zum Problem der biologischen Strahlendosis, *Ztschr. f. d. ges. exper. Med.*, **42**:159-71, 1924.
 52. REIN, H. Experimentelle Studien über Elektroendosmose an überlebender menschlicher Haut, *Ztschr. f. Biol.*, **81**:125-40, 1924.
 53. ———. Zur Elektrophysiologie der menschlichen Haut, *ibid.*, **84**:41-50, 1925.
 54. ———. Die Gleichstromleitereigenschaften und elektromotorischen Kräfte der menschlichen Haut und ihre Auswertung zur Untersuchung von Funktionszuständen des Organes. I-IV, *ibid.*, **85**:195-247, 1926.
 55. ———. Die Elektrophysiologie der Haut. In: JADASSOHN, *Handb. d. Haut- u. Geschlechtskr.*, **1/2**:43-91, 1929.
 56. RICHTER, C. P. The sweat glands studied by the electrical resistance method, *Am. J. Physiol.*, **68**: 147, 1924.
 57. ———. The significance of changes in the electrical resistance of the body during sleep, *Proc. Nat. Acad. Sc.*, **12**:214-22, 1926.
 58. ———. Physiological factors involved in the electric resistance of the skin, *Am. J. Physiol.*, **88**:596-615, 1929.
 59. ———. Pathological sleep and similar conditions studied by electrical skin-resistance method, *Arch. Neurol. & Psychiat.*, **21**:363-75, 1929.
 60. ———. Galvanic skin reflex from animals with complete transection of the spinal cord, *Am. J. Physiol.*, **93**:468-72, 1930.
 61. ———. Sleep produced by hypnotics studied by the electrical skin resistance method, *J. Pharmacol. & Exper. Therap.*, **42**:471-86, 1931.
 62. ———. Instructions for using the cutaneous resistance recorder, or "dermometer," on peripheral nerve injuries, sympathectomies and paravertebral blocks, *J. Neurosurg.*, **3**:181-91, 1946 (see references there).
 63. RICHTER, C. P., and WOODRUFF, B. G. Facial patterns of electric skin resistance. Their relation to sleep, external temperature, hair distribution, sensory dermatomes and skin disease, *Bull. Johns Hopkins Hosp.*, **70**:442-59, 1942.
 64. RICHTER, C. P.; WOODRUFF, B. G.; and EATON, B. C. Hand and foot patterns of low electrical resistance, *J. Neurophysiol.*, **6**: 417-24, 1943.

65. ROSENDAL, T. The conducting properties of the human organism to alternating current. Copenhagen: Munksgaard, 1940 (thesis).
66. ———. Studies on the conducting properties of the human skin to direct current, *Acta physiol. Scandinav.*, **5**:130–51, 1943.
67. ———. Further studies on the conducting properties of human skin to direct and alternating current, *ibid.*, **8**:183–202, 1944.
68. ———. Concluding studies on the conducting properties of human skin to alternating current, *ibid.*, **9**:39–49, 1945.
69. ROST, G. A., and KELLER, P. Die Wirkungen des Lichtes auf die gesunde und kranke Haut. Veränderungen der Membrandurchlässigkeit der Haut nach UV-Bestrahlungen. *In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr.*, **5**/2:89–91, 1929.
70. ROTHMAN, S. Die Resorption durch die Haut. *In: BETHE, Handb. d. norm. u. path. Physiol.*, **4**:107–51, 1929.
71. SCHADE, H., and MARCHIONINI, A. Ueber die Azidose auf der normalen Haut und ihre Bedeutung zur Abwehr der Bakterien, *München. med. Wchnschr.*, **74**:1435–36, 1927.
72. SCHLOSBERG, H., and STANLEY, W. C. A simple test of the normality of twenty-four distributions of electrical skin conductance, *Science*, **117**:35–37, 1953.
73. SCHWARTZ, H. G. Effect of experimental lesions of the cortex on the psychogalvanic reflex of the cat, *Arch. Neurol. & Psychiat.*, **38**:308–20, 1937.
74. TARCHANOFF, J. Über die galvanischen Erscheinungen in der Haut des Menschen bei Reizungen der Sinnesorgane und bei verschiedenen Formen der psychischen Tätigkeit, *Arch. f. d. ges. Physiol.*, **46**:46–55, 1890.
75. VERAGUTH, O. Das psycho-galvanische Reflexphänomen. Berlin: S. Karger, 1909.
76. WAGNER, H. N., JR. Electrical skin resistance studies in two persons with congenital absence of sweat glands, *Arch. Dermat. & Syph.*, **65**:543–48, 1952.
77. WHEELAN, F. G. An instrument for use in measuring electrical resistance of the skin, *Science*, **111**:496–97, 1950.
78. WOEBER, K. Spannungsmessungen an Geweben, *Strahlentherapie*, **77**:265–78, 1948.
79. WOEBER, K., and HOGREBE, H. Spannungsmessungen an Geweben, *Strahlentherapie*, **76**:468–71, 1947.

CHAPTER 3

Percutaneous Absorption

I. TERMINOLOGY	27
II. HISTORICAL	27
III. THEORIES OF TRANSEPIDERMAL ABSORPTION	27
A. Membrane Theory	27
B. Lipid Theory	29
IV. TRANSEPIDERMAL ABSORPTION BEYOND THE SUPERFICIAL BARRIER	29
V. ROLE OF THE LIPID SURFACE FILM	31
VI. ROLE OF THE HORNY LAYER	31
VII. ROLE OF INCREASED BLOOD FLOW	32
VIII. ABSORPTION THROUGH APPENDAGES	33
A. Pilosebaceous Apparatus	33
B. Sweat Ducts	33
IX. PRINCIPLES UNDERLYING METHODS	34
X. ABSORPTION OF SINGLE COMPOUNDS AND GROUPS OF COMPOUNDS	35
A. Water	35
B. Electrolytes	36
C. Lipid-soluble Substances	37
D. Phenolic Compounds	39
E. Hormones	41
F. Vitamins	41
G. Organic Bases	42
H. Heavy Metals and Their Salts	42
I. Fatty Substances	43
J. Water-soluble, Lipid-insoluble Nonelectrolytes	44
K. Gases	44
XI. PRACTICABILITY OF PERCUTANEOUS ADMINISTRATION	46
XII. FACTORS PROMOTING PERCUTANEOUS ABSORPTION	46
A. Effect of Vehicles	47
B. Oil-in-Water Emulsions	48
C. Organic Solvents and Essential Oils	49
D. Physiological Stimulation	49
E. Electrophoresis	50
F. Effect of Concentration	51
G. Effect of Moisture	52

AMONG all aspects of the physiology of the skin, percutaneous absorption has by far the most extensive literature, obviously because of its widespread implications in different branches of medicine, such as physiology, pharmacology, toxicology, in-

dustrial hygiene, balneology, topical and systemic therapeutics, and forensic medicine. Relatively recent review articles (20, 123, 22, 161) published in the last twelve years give us a fair picture of our present knowledge in this field.

I. TERMINOLOGY

"Percutaneous absorption" can be defined as the penetration of substances from the outside into the skin and through the skin into the blood stream. Laug *et al.* (22) discussed the terminology of this process and came to the conclusion that neither of the usual terms is satisfactory: "permeability" connotes to some readers a process of purely physical transfer; "absorption" conveys the sense of cellular activity; and "penetration" has the emphasis on the substance and on its intrinsic properties which enable it to enter or overcome a barrier, no matter whether this barrier is a living or a dead membrane.

Admittedly, we should know more about the role of physiological forces as contrasted with the physical properties of the skin in regulating absorption than we actually do. But even if we knew more, it would be difficult to give preference to any of these terms.

The term "permeability," as it is used in capillary physiology, for instance, includes physiological changes of the membrane, and, conversely, "absorption" depends on the physical properties of the membrane and does not necessarily imply cellular activity.

The word "percutaneous" indicates that transfer takes place through the whole thickness of the skin, and thus it differentiates our topic from "absorption within the skin" as it is dealt with in the huge literature of the Aldrich-McClure test (122). The latter deals exclusively with the disappearance from the skin of substances that have been deposited in the corium, preferably by intradermal injections. Absorption from this site depends on the state of the corium, mainly of the ground substance (chap. 18), blood, and lymph vessels, whereas in percutaneous absorption the state of the epidermis and pilosebaceous apparatus is the decisive factor.

II. HISTORICAL (122, 22)

Up to Fleischer's work in 1877, the view prevailed that human skin is permeable only to volatile substances and gases. Fleischer concluded from his own experiments and from a critical appraisal of older observations that human skin and skin of other mammals was absolutely impermeable to all substances, even to gases. Systematic

experimentation set in at the turn of the century, mainly through the work of Schwenkenbecher (130). His experiments revealed that the skin is permeable to substances which are soluble in lipids and to substances in the gaseous state but is practically impermeable to water and electrolytes.

III. THEORIES OF TRANSEPIDERMAL ABSORPTION

A. MEMBRANE THEORY

In the previous chapter (pp. 10 ff.) the work of H. Rein was dealt with in detail. This author concluded that electrolytes cannot pass through a single layer between cornified and noncornified epidermis ("stra-

tum lucidum"), because this layer is impermeable to anions. Cations, if differences in concentration require, tend to move inward across the membrane but will be held by electrostatic forces. Thereupon, an electrical double layer develops, which is the

site of an electromotive force. It has been pointed out (122) that under natural conditions this double layer, situated between the strongly acid stratum corneum and the mildly alkaline Malpighian layer, has positive hydrogen ions on one side and negative

The theory of pores in the cell membrane (p. 10) explains most satisfactorily how a membrane can be impermeable to either cations or anions. Membranes with extremely fine pores selectively absorb either cations or anions, depending on their electric

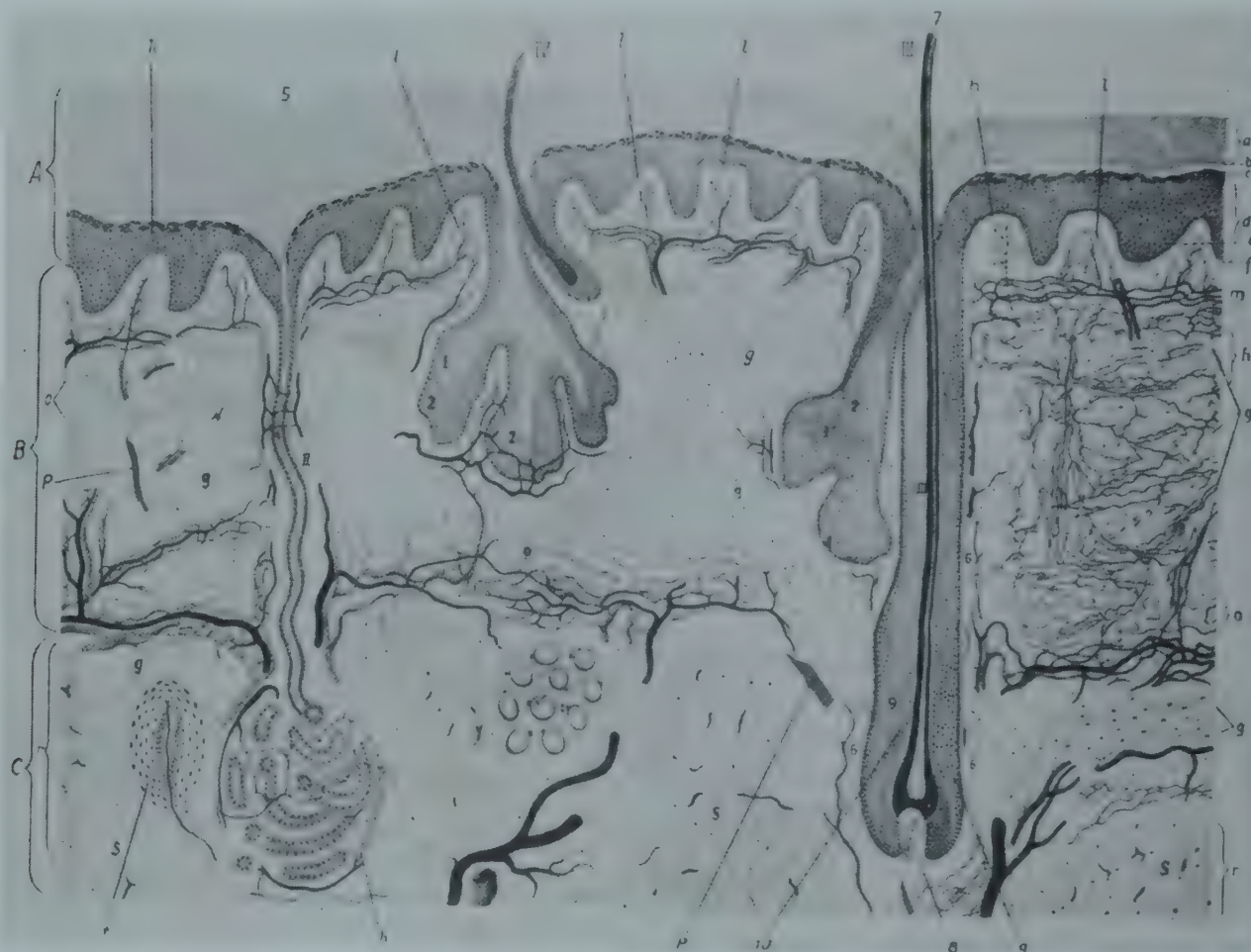


FIG. 1.—Scheme of cross-section of human skin. A, epidermis; B, dermis; C, subdermis; a, horny layer; b, stratum lucidum; c, granular layer; d-e, mucous layer; f, basal layer; g, collagenous fibers; h, elastic fibers; i, papillary capillaries; I, sebaceous gland; II, eccrine sweat gland; III, terminal hair with small sebaceous gland; IV, downy hair with large sebaceous gland; 1, basal layer of sebaceous gland; 2, sebaceous-gland cells; 4, sweat-gland duct with capillaries; 5, sweat-duct pore; 7, hair; 9, external root sheath of hair; 10, internal root sheath of hair (transitorial layer of hair follicle). From Rothman (122). (Drawing by B. Keilitz, Chromolith. Urban & Schwarzenberg, Vienna. Reproduced by permission of Springer-Verlag.)

hydroxyl ions on the other. According to Rein, the horny layer cannot be regarded as a "membrane" because it represents a rather porous, coarse network of horny fibers and is easily penetrated not only by ions but also by large molecules and molecule aggregates; the membrane must be situated below the horny layer.

charge, and therefore these "ion-sieves" are impermeable to electrolytes. Tiny canals in the cell surface may correspond with such pores. Transitional layers of the skin adsorb anions (e.g., Cl^-), whereas cations (e.g., Na^+ , K^+ , H^+ , etc.) may wander a short distance.

Rein's identification of the barrier as be-

ing located in the stratum lucidum has led to some misunderstanding (17) (see also p. 12). Microscopically, the stratum lucidum cannot be seen with sufficient clarity except in the skin of palms and soles, and there is some question whether it is demonstrable at all in other parts (Fig. 1). Therefore, it was proposed (122) that the "transitional layers between cornified and non-cornified epidermis" should be regarded as the site of impermeability for electrolytes, without specifying as to whether this is the stratum granulosum, the stratum lucidum, or even includes the "infrabasal horny layer" (see p. 32).

The same transitional layers have been regarded as barriers to the penetration of water. The hypothesis was advanced (122) that the hydrogen-ion concentration of the proteins in these layers is at the isoelectric point and therefore that these proteins are unable to swell in water. This would imply impermeability to water. It was pointed out (122) that the skin of the frog, which has no transitional layers, is freely permeable to water. In Amphibia, swelling of the whole skin can be observed when, for instance, water is taken up percutaneously by dehydrated frogs (122). In mammals only the horny layer swells perceptibly.

IV. TRANSEPIDERMAL ABSORPTION BEYOND THE SUPERFICIAL BARRIER

Nothing is known about absorption within the Malpighian layer itself. So far, histochemical observations, including the use of isotopes with radiographic technic, indicate that, once a substance coming from the outside has passed the superficial barrier, the epidermal cells do not prevent its further penetration throughout the epidermis. This means that the superficial barrier is quite generally less permeable than the cell membranes within the rete. The intercellular canal system does not seem to play a prominent role in transporting substances downward (122); if such transport takes place, the cells are diffusely penetrated. The same

B. LIPID THEORY

The lipid theory of Meyer and Overton (111) postulates that the cell membrane consists of lipids, because only lipid-soluble substances may penetrate into the cell and their penetration depends on the degree of their solubility in lipids. This theory was based mainly on the easy absorption of alcohols, aldehydes, ketones, and lipid-soluble narcotics. However, many exceptions to this rule were found, and a mosaic theory was presented (108), describing the cell membrane as a mosaic of lipid and protein particles. According to this theory, the proteins may take up water, and the swollen membrane with higher water content may become permeable to water-soluble substances. The mosaic theory did not explain all the facts either, especially since no evidence could be presented that membranes of normal epidermal cells are able to swell in water as long as physiological conditions prevail. However, it seems to be true that the ratio of the solubilities in water and in lipids, as originally postulated in the Meyer-Overton theory, is actually important for the absorption of substances through the skin. The lipid theory was critically reviewed by M. M. Jacobs (70), who amplified it by relating the solubility in lipids to the nonpolar structure of organic molecules.

picture of diffuse penetration of the Malpighian layer is obtained when material enters the epidermis from below or from the side after breaking through the follicular wall or through the basement membrane of the sebaceous gland, as is observed in trans-follicular absorption and most clearly shown by the work of MacKee, Sulzberger, Herrmann, and Baer (93) (Fig. 2). A single exception to this diffuse penetration was reported by Moncorps (107), who found traces of mercury in the intercellular spaces of the epidermis after inunction with ammoniated mercury ointment.

For some substances there might be a

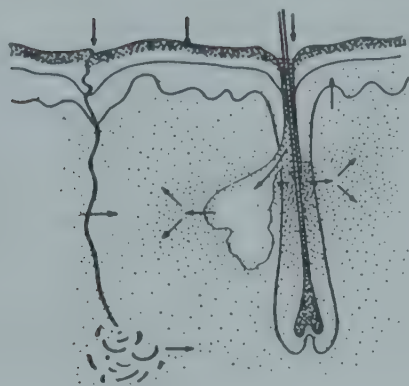


FIG. 2.—Schematic presentation of probable routes of penetration and the distribution of tracer substances, using as vehicles mixtures of various solvents, interface active agents, coupling agents and solubilizers. Arrows indicate probable routes of penetration and spread; dotted areas indicate deposit of material. From MacKee *et al.* (93). (Reproduced by permission of the Williams & Wilkins Company.)

barrier at the epidermal-dermal boundary. Ferguson and Silver (44), using a chlorine-liberating ointment, found, by means of an orthotolidine reagent, free chlorine throughout all layers of the epidermis but no penetration into the corium beyond the "basement membrane." A similar pattern was found by Cullumbine (29) for the penetration of mustard gas into the skin of man and guinea pigs (but not of goat and rabbit). If the basement membrane is actually a barrier, it may happen that some such substances do not enter the papillary capillaries, because the capillary wall is clearly beyond the lower surface of the basement membrane. In the majority of cases it seems, however, that, once a substance passes the superficial barrier and the rete, it will be

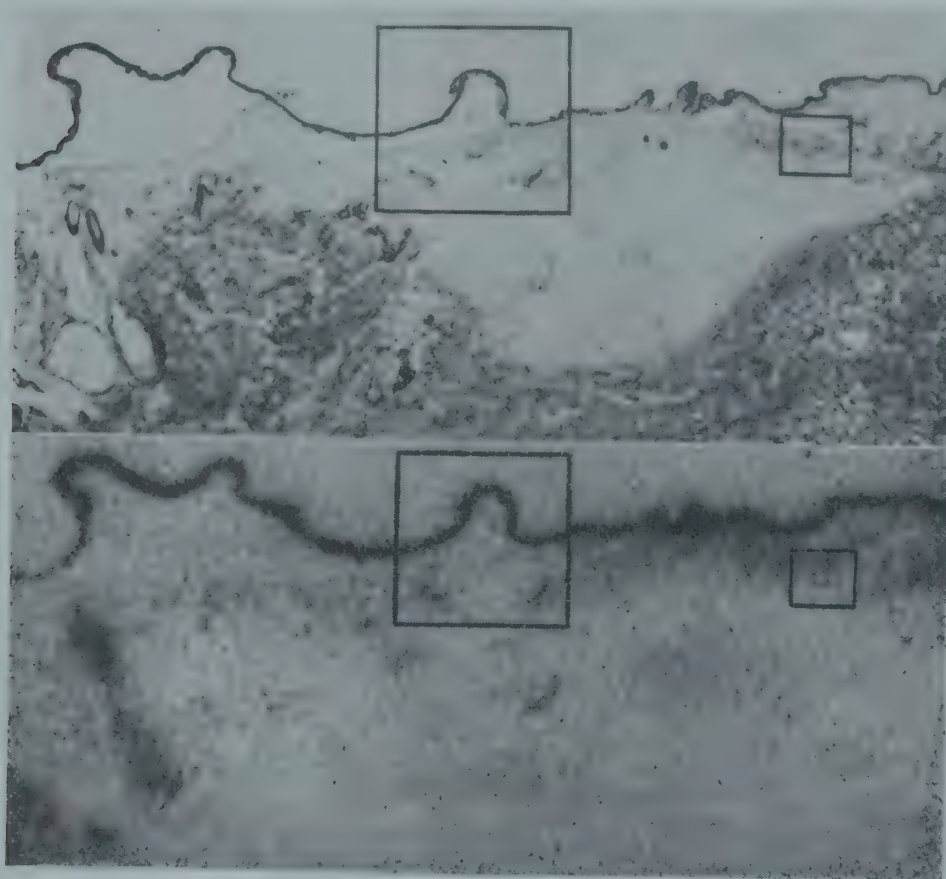


FIG. 3. Human skin exposed for 15 minutes to 475 γ of radioactive lewisite. Tissue excised 24 hours after application. Fixation occurs primarily in hair follicles and sebaceous glands (at extreme left of photomicrograph) and in the epidermis. The squared areas show blood vessels which have accumulated small amounts of activity. From Axelrod and Hamilton (9). (Reproduced by permission of the American Association of Pathologists and Bacteriologists.)

able to enter the circulation through the capillary endothelium. Thus, for instance, lewisite, which in human skin is fixed almost

exclusively in the epidermis and in follicles, can still be demonstrated in blood vessels in small amounts (9) (Fig. 3).

V. ROLE OF THE LIPID SURFACE FILM

The surface of the skin is covered with a waxy layer (p. 309), whose role in the impermeability of the skin to water and electrolytes has been overestimated. It is true that it hinders the wetting of the cornified surface and delays the penetration of water and aqueous solutions into the horny layer. But the waxy film is miscible with water, because it contains cholesterol, cholesterol esters, and waxes, which are good emulsifiers. Therefore, after prolonged contact with water, particularly with warm water, the permeation of aqueous solutions becomes possible, and the horny layer swells. If wet dressings are applied to the skin with a water-impermeable cover, this swelling is grossly seen, the horny layer showing abnormal wrinkling and whitish discoloration ("maceration"). After a prolonged warm bath this effect is well seen on palms and

soles, not because of the lack of a waxy cover but because of the greater thickness of the horny layer. Of course, fat-soluble materials may be retained for a while in a fatty layer on top of the skin. If the material to be absorbed is volatile, considerably smaller amounts may come to absorption because of the temporary retention on the surface and evaporation in the meanwhile. Cullumbine (30) showed that this is the case with mustard gas when the skin is covered with anhydrous wool fat. Conversely, the vesicant action of mustard gas may be increased if the skin is defatted prior to its application. This was achieved with xylol but not with ether. Laug *et al.* (83), studying absorption of mercury from ointments, found that washing the skin with soap and water prior to the ointment application had no effect.

VI. ROLE OF THE HORNY LAYER

As with the greasy cover, the role of the horny layer has been misinterpreted as a barrier to percutaneous absorption. As mentioned above, this layer has large pores and is permeated even by gross molecule aggregates. It is true that some material may be adsorbed on the surface of the horny fibers; the dyeing of wool and fur is based on such adsorptive processes. But, to conclude from staining experiments, such adsorption in the horny layer is very limited, the surfaces become saturated after the uptake of minute amounts, and the meshes between the fibers are filled with material.

If the horny layer were of decisive influence, one would expect decreased absorptivity when the horny layer is thickened. In ichthyosis vulgaris, however, in which the skin differs from normal only by a thickened horny layer, the percutaneous absorption was found to be normal (110). The situation

may be different in the case of qualitatively "superkeratinized" material (p. 378) as it occurs in corns and calluses. No experimental data are available on absorption through such pathological lesions. Still it is remarkable that nail plates, which are certainly "hyperkeratinized" as compared with the epidermal horny layer, are easily permeated by water (18) and, in my experience, also by water-soluble anilin dyes.

It is not quite clear whether the naturally thick horny layers of palms and soles are penetrated as well as the horny layer of other parts. Miescher (98) found that water-soluble fluorescent dyes did not entirely penetrate the horny layer of the cat's paw: only the upper loose strata were diffusely stained; nevertheless, the dye could be seen in the depth of the horny layer in single slit-like spaces. Otherwise, it was often found that there is less absorption through palms

and soles than through other parts of the surface. However, this was attributed to the absence of hair follicles rather than to the thickness of the horny layer (122).

According to quite a few histological data, the lowest compact part of the horny layer is different from the looser strata above, and it might be justifiable to consider whether it should not be included in the concept of "transitional layers." According to Unna (152), this lowest part of the stratum corneum has a thickness of 5 to 6 cell layers and can be subdivided into (a) an infrabasal layer containing glycogen and (b) a basal horny layer containing eleidin and a substance oxidizable by osmic acid. Even outside palms and soles, the lower part of the horny layer sometimes behaves differently from the upper part. MacKee *et al.* (93), for instance, describe the barrier in man (see p. 29) as including "the lowest portion of the stratum corneum plus the stratum lucidum and stratum granulosum."

It is generally assumed that loosening of the horny layer by so-called "keratolytic

agents" facilitates penetration. Salicylic acid, for instance, is used in topical treatment of hyperkeratotic lesions, to facilitate the penetration of sulfur, ammoniated mercury, etc. Milbradt (99) demonstrated histologically that salicylic acid, pyrogallol, resorcinol, and β -naphthol, all regarded as keratolytics, greatly enhance the penetration of dyes.

Whereas in my original review (122) I stated that mild keratolytic agents cannot promote percutaneous absorption as long as they do not attack the "barrier," Laug (22) pointed out how difficult it is to conceive that keratolytic action should be sharply limited to the surface layer of cornified cells. Indeed, it has been shown that salicylic acid is not purely keratolytic but rather readily causes "rete-cytolysis," with destruction of the barrier (142). Even under mild conditions, when low concentrations are used and there is no airtight closure, the cell membranes of the transitional layers may be attacked, no matter whether this can be demonstrated histologically or not.

VII. ROLE OF INCREASED BLOOD FLOW

Increased blood flow through the corium does not necessarily increase penetration. It is true that, once a substance has reached the vascular bed, its rate of removal is greater if the blood flow is increased. In the case of most substances in gaseous form, for instance, there is a simple physical diffusion through the skin, depending on the difference in concentration (or vapor tension) of the gas outside and that inside the skin and on the temperature. If the concentration is greater on the outside, so that the diffusion is directed inward, a greater difference will be maintained by more rapid removal of the substance from the spot where it entered the circulation. For instance, carbon dioxide dissolved in water passes through the skin inward if its outside concentration is higher than 8.5 per cent (77). In its passage it reddens the skin independently of the temperature of the bath. Owing to the dilatation of arterial and venous limbs of superficial

capillaries, there is a significant and prolonged increase in blood flow (141). This carbon dioxide hyperemia may promote the absorption of another gas through the skin; McClellan *et al.* (92) have shown that radon penetrates much faster through the skin from baths containing carbon dioxide than from plain water. Absorption of nitrogen and of helium gas also could be related to the rate of peripheral blood flow (10).¹

However, pure vasodilatation without epidermal changes is a rather uncommon situation. In the majority of cases when the skin becomes reddened, it is because it is "irritated"; in irritated skin the epidermal cells are damaged and their permeability increased. The true barrier lies above the capillary bed; and if a substance does not penetrate this barrier, hyperemia cannot be expected to promote its absorption. If a

1. See also absorption of tritium oxide, p. 35.

substance penetrates only after mechanical, physical, or chemical stimulation of the skin, this is because the epidermal barrier has been damaged.

It has been a rather unique observation

that irradiation with red and short-wave infrared rays promotes subsequent absorption of penicillin from wet compresses (31). Whether this is due to hyperemia or to some other factor has not been investigated.

VIII. ABSORPTION THROUGH APPENDAGES

The mechanism of percutaneous absorption as a whole becomes somewhat complicated by the presence in the skin of gland ducts and hair follicles which open on the surface in the form of visible pores.

A. PILOSEBACEOUS APPARATUS

Older data (123) as well as modern work (146) have shown the prominent role of transfollicular absorption. In the upper half of the follicular canal the hair shaft does not adhere to the follicular wall. The space between is loosely filled with greasy horny scales and contains air. This space is continuous with the duct of the sebaceous gland, which empties the sebum into it (see Fig. 1, p. 28). Any substance may penetrate this space and reach the duct of the sebaceous glands, and from there the gland cells (see Fig. 2, p. 30). This pathway does not require passage through a stratified epithelium but leads through an air-filled canal to cell membranes, the permeability of which is considerably higher than that of the transitional epidermal cells. Similarly, the side walls of the follicles are less resistant to penetration than is the surface epidermis. This was first shown with dyes in electrophoretic experiments by Rein (116). He found that if the rete takes up any stain, the vicinity of follicles is stained most intensely. He also found that perifollicular staining does not occur around follicles which have been occluded by waxy plugs. This type of penetration was most extensively proved by MacKee *et al.* (93). These authors studied the penetration of heavy-metal compounds, sulfonamides, and dyes through the skin of guinea pigs and men. The best penetration was obtained by the use of vehicles combining water, propylene glycol, wetting agents, "coupling agents" (antipyrine), and solubil-

izers. With these vehicles, staining of the follicles, epidermis, and corium was achieved. However, in all cases a band free of color was observed in the transitional area. In their interpretation the authors outlined the main route of penetration as follows: horny layer → follicular pores → follicles → corium via both sebaceous glands and side walls of follicles → spread in corium downward, to the side, and up into the epidermis. According to this scheme, the epidermal barrier is not broken through, either from above or from below (see Fig. 2, p. 30).

Water and aqueous solutions do not enter the follicles by mere capillary attraction because of insufficient wetting and because the ducts are filled with air. Under pressure, however, the adherent air may separate and be forced out to the surface. In the case of inunction of ointments or of vehicles containing wetting agents, compression of the air in the duct and absorption of the air through gland cells may take place (123).

B. SWEAT DUCTS

The pores of sweat ducts, like those of follicles, become stained if dyes are introduced into the human skin by electrophoresis (116). Ichihashi (68) postulated that this "pore pattern" indicated absorption of the dye through the sweat ducts. Abramson *et al.* (4, 3, 2), came to the same conclusion and found the development of the sweat pore patterns dependent on the electric charge of the skin. However, Flesch *et al.* (45) showed that the pore pattern of sweat pores was readily erased by the scraping-off of the horny layer with a scalpel and that penetration of the dye into the duct could not be demonstrated histologically. These observations did not definitely disprove penetration through sweat glands: the dyes

might have been reduced in the duct to a leuko-form; but they clearly indicated that palmar "pore patterns" were insufficient evidence for transudoriferous absorption.

In any case, the poor absorptive capacity of palms and soles (p. 31) militates against any substantial penetration through sweat ducts. Poor penetration of dyes through

palms and soles was noted by MacKee *et al.* (93); of allergens, by Herrmann (63); and of histamine, by Shelley and Melton (136).

It has been known since the last century that metallic mercury, which easily passes through the pilosebaceous apparatus, can be seen in sweat orifices, rarely in a sweat canal, and never in a sweat gland.

IX. PRINCIPLES UNDERLYING METHODS

In experiments on percutaneous absorption the intake of substances through the respiratory tract and through mucous membranes of mouth, rectum, and vagina has to be carefully avoided. Working with volatile substances of relatively high vapor pressure, one must be particularly cautious to avoid errors in attributing absorption through the lungs to percutaneous penetration. Another elementary rule for experimentation in this field is that the skin through which penetration is tested must be absolutely intact. Many erroneous conclusions were drawn from animal experiments in which the skin was shaved or otherwise depilated, without the necessary care to avoid injury to the rather superficially situated barrier.

Whether a substance is absorbed through the skin or not has been most commonly tested by attempting to demonstrate the substance chemically in the blood, urine, internal organs, or the expired air. Or the penetration has been detected by the local or systemic biological, pharmacological, or toxic action of the material. The absorption of small quantities may remain undetected with any of these methods, because of the limited sensitivity of the assay. Furthermore, these methods can hardly be used to demonstrate percutaneous absorption of substances normally present in the body. To work with the latter ones, Hediger (62) introduced a new method which in a modified form was widely and fruitfully used by Bürgi (20). In this method the substances to be tested are applied in solutions under glass bells which are affixed to the skin. Changes in the concentration of the dissolved substances are estimated at regular time inter-

vals in aliquot parts by chemical analysis of the fluid above the skin. Decrease of concentration indicates absorption of the solute. The time curve of rate of absorption can be well established with this method. Again, this method has its limitations because of the sensitivity limit of the chemical method used. In addition, as pointed out by Laug (22), deposition in the horny layer and in follicular openings may simulate true penetration when there is none. But such surface deposition is limited, and if the decrease in concentration is substantial and continuous, there can be little doubt that the substance does penetrate.

One of the most sensitive tests is that based on the antigenic properties of the substance to be absorbed, because extremely minute traces penetrating to the site of tissue antibodies may suffice to elicit allergic reactions.

Histochemical methods of demonstrating the penetration of substances into the skin have the advantage of revealing not only absorption per se but also the route of penetration. However, this method is limited to dyes and to a rather small number of other substances which yield precipitable colored end-products in specific chemical reactions. A dangerous error may arise in the use of dyes as "indicators" by mixing them with noncolored substances and then arriving at conclusions concerning the absorption of the noncolored material. Separation within the skin of dyed substance and dye is generally the rule; only exceptionally will they stay together.

Radioisotope tracer technics are obviously far superior in all respects to all pre-

vious methods in the study of percutaneous absorption. These methods enable us to study qualitatively and quantitatively the absorption of minute traces of normal constituents and of foreign substances by measuring radioactivity in the skin, blood, urine,

internal organs, and expired air. Moreover, by means of the autoradiograph technic one can follow the pathway of virtually all materials. We are just beginning to utilize isotopes for this purpose, with some remarkable initial results.

X. ABSORPTION OF SINGLE COMPOUNDS AND GROUPS OF COMPOUNDS

A. WATER

Obsolete plethysmographic work yielded the first experimental indication that human skin is either impermeable to water from the outside or that if there is any absorption, the amount absorbed is very small (122). This conclusion was arrived at also by Schwenkenbecher (130). I pointed out (122) that under physiological conditions the horny layer swells when a limb is kept in water but that the living epidermal cells, as contrasted with the skin of aquatic animals and Amphibia, remain microscopically unchanged, an indication of a barrier of water penetration below the area of swelling (p. 52). I also assumed that, because of this barrier, if there is water penetration at all, it must be through the appendages (122). After prolonged contact, water may displace the air, penetrate into the follicles, and from there, via sebaceous glands or follicular side walls, into the general circulation.

Whitehouse *et al.* (170, 171), carefully estimating body weight before and after immersion in water baths, came to the conclusion that water does penetrate through human skin. But their method has been open to criticism (22), and only recently was the penetration of water definitely proved by the use of heavy water (deuterium oxide) and radioactive water (tritium oxide) (see below). Szczesniak, Sherman, and Harris (149) immersed young male rats for 6–7 hours in a mixture of 6 parts H_2O and 4 parts D_2O at $35^\circ C$. and recovered substantial amounts of deuterium oxide in the heart blood. The amounts were approximately the same in animals whose fur was removed by clipping and by application of an adhesive tape as in animals which were immersed

with their natural fur. The authors did not attempt to trace the route of absorption by use of tritium oxide and autoradiography (personal communication); and thus it is still not decided whether this absorption is transepidermal or via appendages.

It may appear paradoxical to assume that there is no transepidermal inward movement of water, as it is well known that water continuously moves outward through the epidermis (p. 239). However, one-sided "irreciprocal" permeability of membranes is a well-known phenomenon in biology. Wertheimer (168, 169) studied many instances of this irreciprocal permeability of frog's skin and showed that, by changing the experimental conditions, the frog's skin can be made permeable to water either inward or outward by changing the electrical charge of the skin membrane by the addition of weak acids, alkalies, and salt solutions.²

Assumption of irreciprocal permeability to water, however, is certainly unnecessary in the light of the recent work of Pinson (E. A. Pinson, Water exchanges and barriers as studied by the use of hydrogen isotopes, *Physiol. Rev.*, **32**:123–34, 1952). Exposing

2. Wertheimer (167) also demonstrated similar one-sided permeability to electrolytes. Under natural conditions sodium chloride penetrates from the outside through frog skin, but not in the opposite direction. This migration is due to the potential difference between the outer and inner surfaces of the skin and can be modified by changing the potential. Wertheimer's work was confirmed and expanded by modern isotope technics (154, 48, 155, 67, 66, 90, 128). Ussing (154) demonstrated that the reciprocal permeabilities of Cl^- and Na^+ ions are different and independent of each other. It appears that hormonal influences on the permeability of the skin to water and electrolytes also depend on the electrical charge (48, 128).

a skin area of man to an outside environment saturated with tritium oxide vapor, he found a passage inward through the skin approximately at the same rate at which water passes out through the skin normally as insensible perspiration (pp. 233ff.). Pinson obtained similar results when he immersed his skin in a solution of tritium oxide. In either case, when the radioactive water was applied as a vapor or as a solution, it took about 10 minutes from the beginning of the exposure for the tritium oxide to appear in the urine. Excretion increased for from 30 minutes to several hours. If the skin was warmed after exposure, the concentration of tritium oxide in the urine increased rapidly and reached a constant value in about 1 hour. In case of cooling after exposure, the concentration of tritium oxide in the urine increased slowly for a period longer than 5 hours.

Pinson assumed that tritium oxide penetrates the skin barrier in vapor form. This assumption explained that it made no difference whether vapor or solution was applied and that warming the skin had an accelerating effect. But he also thought that, in addition to the superficial barrier, there might be a deeper one "between the skin and the general circulation." This second barrier might be influenced by vasoconstriction, hence the slowing-down effect of cold.

One might argue that the structure of tritium oxide differs quite considerably from that of water and therefore that no conclusions can be drawn concerning the absorption of water from experiments with tritium oxide. Pinson has discussed this point adequately. In any case, the concept that there is diffusion of water vapor inward and outward in equal amounts is a novel and intriguing interpretation, particularly because it is known that any substance may pass the skin barrier in the gaseous state (p. 44).

B. ELECTROLYTES

Up to recent times the sum of experimental data led to the conclusion that electrolytes applied to the skin in aqueous solutions either do not penetrate the skin of

mammals, or, if they enter, they do in small amounts via the appendages. When salts with nonphysiologic cations, such as Li, Rb, Cs, Sr, and Ba, were applied to the skin in aqueous solutions in the form of baths, sprays, etc., these ions could not be recovered in the urine (122, 74, 171). Lehman (85), using Hediger's method, found the skin impermeable also to the physiologically occurring cations Na and Ca when applied to the skin in the form of NaCl and CaCl₂ aqueous solutions. Thus, until the advent of the isotope research technics, passage of cations never was demonstrated or even claimed to occur.

Of the anions, the iodide ion was tested most frequently, and with this ion the results were not unequivocally negative. In a number of experiments small amounts of iodides were recovered in the urine after iodides had been applied to the skin in aqueous solutions. The iodide ion has a relatively good chance to enter the skin, in comparison with chloride, because it is an anion which increases the negative electric charge of the skin and its capacity for swelling in water (122). Also there is some evidence that small amounts of iodides may be oxidized to free iodine in the skin and be absorbed in this form (122, 58). But absorption of chlorides in small amounts also has been reported in a few publications. Antinobin (8) found small amounts absorbed when the hands were immersed in N/1 sodium chloride solution by "remainder analysis," whereas with a similar method Bürgi (20) found traces absorbed only from highly concentrated solutions (10–27 per cent).

Sixty years ago Traube-Mengarini (151) reported that iodides penetrate via the appendages. Her findings have never been checked, but on the basis of Rein's work (p. 33) we must assume that the small amounts of electrolytes which may penetrate do so via the appendages and not transepidermally. It seems to be well established that in the epidermis there is a rather superficially located barrier preventing the penetration of electrolytes. If this barrier is broken by very superficial injuries to the

skin or by natural disease processes, the skin becomes freely permeable to electrolytes. Recently, considerable absorption of sodium ions from sodium bicarbonate baths was demonstrated in cases of exfoliative dermatitis (43).

With the isotope tracer technic two positive findings are recorded on percutaneous penetration of electrolytes in man. Johnston and Lee (73) used aqueous solutions of sodium chloride containing radioactive sodium. They incorporated the solutions in different ointment bases and rubbed these ointments thoroughly into the right upper arm of normal subjects. Absorption was checked by measuring radioactivity on the left hand and in the urine. Activity was demonstrated in both these places. Conspicuously, the activity was greatest very soon after the application and rapidly decreased to minute intensities, suggesting that the mechanical factor of pressing the ointment into the skin caused the absorption, whereas no absorption, or only a trace, took place from the ointment when it was left on the skin without pressure.

Potassium iodide solutions with I^{131} were applied to human skin by O. B. Miller and Selle (101). Radioautographs suggested accumulation of radioiodine around the hair follicles; but the pictures are not clear enough to be sure that the radioiodine passed the cornified parts of epidermis and follicular openings. The disappearance of radioactivity from the skin surface was studied, and it was found that the disappearance was considerably slowed down if the covering of the area was airtight. Thus the gradual subsidence of activity might have been due not to absorption but to evaporation or to some other kind of "surface loss." Radioactivity of the thyroid gland and of urine was tested in one subject and was found to be absent.

In animals the percutaneous penetration of electrolytes is better evidenced. Loeffler and Thomas (88) studied the percutaneous absorption of aqueous radioactive strontium chloride solutions ($Sr^{89}Cl_2$) through the shaved skin of rats. Slightly over 10 per cent

of the applied material went through the intact skin. The uptake was very rapid, most of it occurring in the first 10 minutes. When the skin was damaged, 50 per cent of the material went through. Once the barrier was broken, the degree and type of damage (abrasions, stab wounds, lacerations) did not make any difference in rate and amount of absorption.

Miller and Selle (101) found percutaneous passage of I^{131} in the form of KI aqueous solutions incorporated in a water-miscible ointment base. When such material was applied to the unabraded skin of pregnant guinea pigs and rabbits and was covered with Scotch tape for 18–24 hours, considerable activity could be demonstrated in the feti. In some cases the radioactivity proved to be fatal (101, 132).

In contrast to these findings, Strohl *et al.* (145), who found considerable activity in the blood after electrophoretic introduction of radioactive sodium iodide into the skin of rats, guinea pigs, and rabbits, state that penetration by "simple diffusion" (meaning application without electric current) is negligible (Fig. 4).

C. LIPID-SOLUBLE SUBSTANCES

The idea that the permeability of the skin to lipid-soluble substances comes about because the cell membranes have a sterol-phosphatide framework was supported in 1936 by Starkenstein and his co-workers (140, 95). They showed that any disturbance of this framework either by dissolution or by precipitation of cholesterol breaks up the barrier to skin absorption. Previous treatment of the skin with chloroform or ether, both of which dissolve cholesterol, or with saponins (100), which precipitate it, makes the skin permeable to water-soluble substances and diminishes the penetration of lipid-soluble compounds. Conversely, the introduction of cholesterol into the skin, for instance with ointments, diminishes the permeability because the cell membrane has been strengthened. In fact, from a table of my early review (122) it is obvious that the absorption of potassium iodide from oint-

ment bases containing cholesterol is somewhat poorer than from bases without it.

In general, lipid-soluble substances are fairly rapidly and completely absorbed through the skin. The absorption appears to be faster if the substance is to some de-

they claim, are insoluble in water. However, they do not state to what degree they are insoluble.

Most substances which are soluble in both lipids and water penetrate so rapidly that the rate of absorption is comparable to

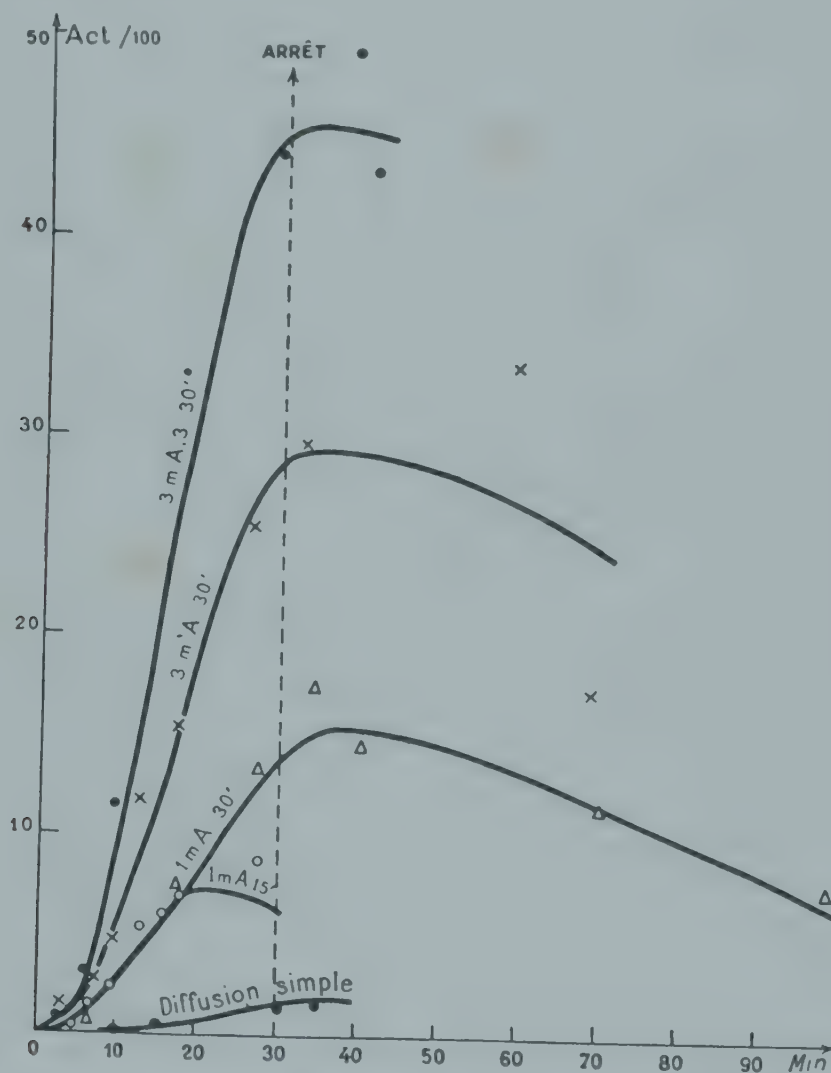


FIG. 4.—Development of activity in the blood of rats as a function of intensity of current and length of application after electrophoretic introduction of radioactive sodium iodide into the skin. From Strohl *et al.* (145). (Reproduced by permission of Dr. Strohl and Masson & Cie.)

gree soluble also in water. In spite of extensive discussion of this point (123, 22, 161, 20), it has not been established that absorption is a straight function of the distribution coefficient lipid-solubility/water-solubility; neither do we know anything about the optimum point of this coefficient. Bischler *et al.* (14) emphatically deny the necessity of solubility in water. They find excellent penetration of oleates of the alkaloids, which,

gastrointestinal absorption or even to routes of parenteral absorption (e.g., subcutaneous injections). This rapidity has left little doubt that the route of absorption is transepidermal. But only in the 1940's was this directly demonstrated for mustard gas by Cullum-bine (29) with a histochemical reaction and by Axelrod and Hamilton (9) by the use of mustard gas labeled with radioactive sulfur (S^{35}) (Fig. 5). Interestingly, lewisite pene-

trates pigskin primarily through hair follicles and apparently spreads from there into epidermis and corium (Fig. 6).

The number of lipid-soluble substances which have been proved to penetrate through the intact skin into the general circulation is too large to be exhaustively listed here. Only a few of the medically important groups of lipid-soluble substances will be discussed, namely, phenolic com-

minish for some time. Many doubted its being dangerous at all. As late as 1939 the question was again discussed as to whether the local application of phenol may cause any systemic effect (123), though many people have died from such treatment. The reason for the uncertainty in a problem settled a long time ago may be found in the fact that phenol in high concentration coagulates the proteins of the skin and com-

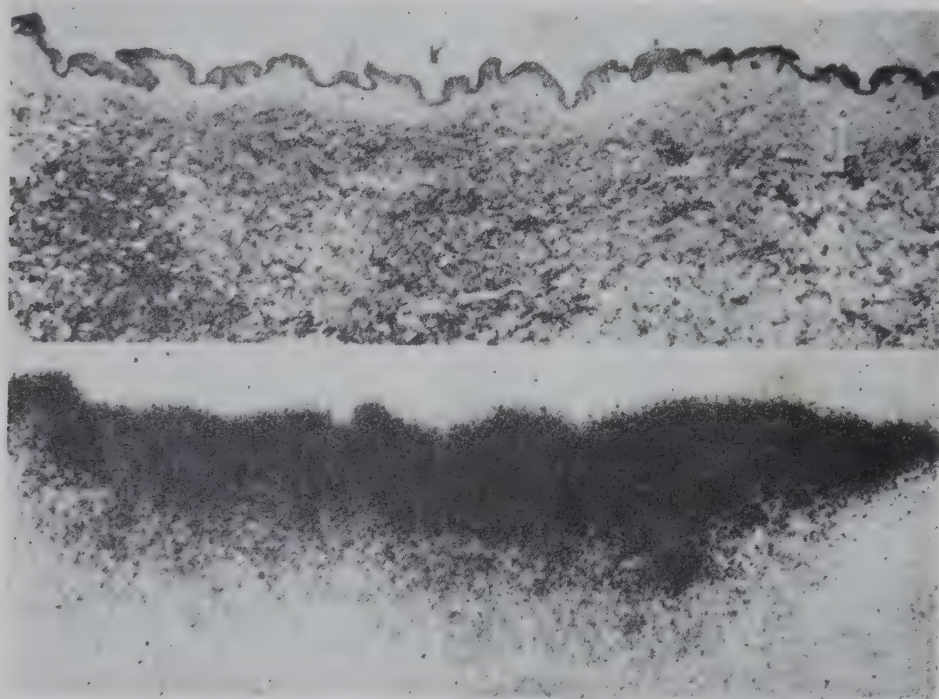


FIG. 5.—Human skin exposed for 10 minutes to 475 γ of radioactive mustard. Tissue excised 24 hours after application. Fixation of mustard occurs in epidermis and corium. From Axelrod and Hamilton (9). (Reproduced by permission of the American Association of Pathologists and Bacteriologists.)

pounds, hormones, vitamins, organic bases, a few pharmacologically or toxicologically important compounds, and fatty ointment bases.

D. PHENOLIC COMPOUNDS

The percutaneous absorption of phenol was well known to physicians of the older generation. They witnessed severe and sometimes fatal poisoning following the application of carbolic acid to large areas of the skin. Such unfortunate events did not occur frequently, however, and the popularity of this kind of treatment did not di-

minish with them to form large molecules, the lipid-solubility and penetration capacity of which are completely different from those of free phenol. It should be stated here as a general rule that any substance when applied in a high concentration having a caustic effect is absorbed in relatively smaller amounts than when the same substance is applied in lower concentrations which do not elicit a gross response. This has been proved not only for phenol (91) but also for a number of heavy-metal salts (91, 19) and for hydrogen sulfide (129). In addition, it has been shown that previous treatment of

the skin with caustics (19) or with astringents (96) decreases the permeability to other absorbable substances.

The percutaneous absorption of phenol and its toxicity were reviewed and thoroughly studied by Deichmann *et al.* (33, 34, 35). These authors confirmed the old claim that the addition of camphor counteracts the toxicity of percutaneously absorbed phenol

Salicylic acid, the most frequently tested substance in experiments on percutaneous absorption, penetrates with extreme ease through the skin from alcoholic or aqueous solutions as well as from ointments. Lipid-solubility is a property of the nondissociated molecule and not of the salicylate ion. Sodium salicylate, which is ionized in aqueous solutions, is not lipid-soluble and does not



FIG. 6.—Pig skin exposed to 1.269 γ of radioactive lewisite for 15 minutes; excised 24 hours later. The bulk of the lewisite is accumulated in hair follicles and hairs, with smaller amounts in the epidermis. From Axelrod and Hamilton (9). (Reproduced by permission of the American Association of Pathologists and Bacteriologists.)

to a measurable degree. A relatively recent fatality due to percutaneous absorption of phenol was reported by Cronin and Bauer (28).

An interesting feature of phenol absorption is that, in spite of its good penetration through intact skin, this can be considerably increased by injuring the skin. Freeman, Alvarez, and Draize (47) found an increase in absorption of 50 per cent after superficial abrasion and of 130 per cent after heat burn of the skin.³

penetrate, or does so only in traces, unless there is enough carbon dioxide in the tissue to liberate salicylic acid from the salt (122, 65). The esters of salicylic acid, used externally as antirheumatic preparations, are lipid-soluble and penetrate easily (122, 16). However, different esters penetrate at dif-

3. A similar situation was found for the lipid- and water-soluble compounds diethylene glycol and 2-methyl-2,4-pentanediol (23). Thus it appears that there exists an, at least relative, barrier also for lipid-soluble substances. It would be desirable to know where it is and what its nature may be.

ferent rates, and it is not known what factors regulate these rates. Halpern *et al.* (59), for instance, find the penetration of diethylamine salicylate nearly three times faster than that of the methyl ester.

Other lipid-soluble derivatives of phenol, such as resorcinol, hydroquinone, and pyrogallol, also enter the skin from any vehicle (161).

E. HORMONES

Estrogenic hormones, testosterone (42, 22, 161), progesterone (69), and desoxycorticosterone (160) penetrate the intact skin rapidly and with great ease. In the end-result it makes little difference whether these hormones are given by injection or through the skin, although the latter route of absorption is somewhat slower. But the uptake is quantitative.

At the present time it is difficult to make an unequivocal statement on the percutaneous absorption of cortisone and hydrocortisone through intact skin. The animal experiments of Castor and Baker (25), who applied cortisone acetate and hydrocortisone acetate to the skin of rats in alcoholic solutions, suggest that these salts do penetrate, since their percutaneous application produces profound morphological changes. In man, however, such changes are not produced with cortisone acetate, and the local application of this compound for therapeutic purposes has been disappointing in all diseases, in which its systemic administration has been most effective (54, 147). It has been debated that possibly cortisone is ineffective in local application because it has to undergo some chemical change before it becomes biologically effective and that the skin is not able to effect this chemical transformation (125). The observation that hydrocortisone, as contrasted with cortisone, is therapeutically effective in percutaneous application (148) seems to support this view. Thus the ineffectiveness of cortisone acetate in local application does not necessarily mean that it is not absorbed.

No work has been done as yet with the free cortisone and hydrocortisone steroid al-

cohols in respect to percutaneous absorption. One would expect them to penetrate with greater ease than their salts.

Data concerning absorption of water-soluble hormones through the skin are highly uncertain. From the extensive work on percutaneous absorption of insulin (123), one can conclude that under carefully controlled experimental conditions negative results are prevalent. Positive results are due either to skin injuries from shaving in animal experiments or to a preparatory treatment of the skin with chemicals which pathologically increase the permeability of the skin (123).

Absorption of iodothyroglobulin through the skin of guinea pigs was concluded by Albrieux (5) from weight loss and increased oxygen uptake by the treated animals. The material was rubbed into "epilated skin," but what kind of epilation was used has not been stated.

Among the water-soluble pituitary hormones, apparently only the lactogenic hormone was tested for its penetration through the skin; it was found to be absorbed in women by Ehrhardt (39) and in guinea pigs by Voss (163). Walker and Rothman (164) also noticed systemic effects of this hormone when it was massaged into bald scalps.

F. VITAMINS

The lipid-soluble vitamins A, D, and K penetrate the skin with ease (42, 22).⁴ The biological significance of vitamin D formation in the skin by ultraviolet light and its subsequent absorption is obvious. Carotenes are also easily absorbed.

Absorption of water-soluble and lipid-insoluble vitamins (particularly of thiamine and of ascorbic acid) has been claimed but not confirmed (123). Lipid-soluble derivatives of these vitamins, however, are absorbed, e.g., pantothenol, the alcohol of pantothenic acid (21).

4. According to P. Flesch, there is no satisfactory evidence for percutaneous absorption of vitamin A in rats and man (personal communication; see also *J. Invest. Dermat.*, 19:353-63, 1952).

G. ORGANIC BASES

Large groups of pharmacologically active agents are organic bases, e.g., alkaloids of plant origin, synthetic antiseptics, analgesics, antihistaminics, etc. The free bases are lipid-soluble and penetrate the skin. Their water-soluble salts do not penetrate or penetrate only in traces through appendages when applied for several hours, whereas the base in the same amount and same concentration, applied over the same surface area, immediately causes pharmacologic effects. Poisonous alkaloid bases when applied to the skin may kill the animals in a few minutes, when the comparable application of the water-soluble salt has no effect at all. For instance, nicotine, strychnine, and opium alkaloids are absorbed with great ease but not their sulfate or hydrochloric acid salts (123). Recently the much greater penetration of histamine base as compared with histamine salts was demonstrated by Shelley and Melton (137).

Modern drug industry has made little use of this quite general rule. Most modern anesthetics, antihistaminics, and bactericidal preparations for topical use contain the water-soluble salts and not the free bases. Of course, all these agents are allergens, and, if the bases were used, with improved absorption the incidence of sensitization might increase considerably (124). The close connection of increased penetration with increased incidence of sensitization was pointed out by Rabeau and Malangeau (114).

H. HEAVY METALS AND THEIR SALTS

Of the lipid-soluble salts of heavy metals, mercuric bichloride was often found to be absorbed from its aqueous solutions through the unbroken skin. Like phenol, however, its penetration is interfered with by its coagulating effect on proteins when applied in concentrations above 0.5 per cent (19); and thus, in spite of its good solubility in organic solvents (in alcohol 33 gm. in 100 ml. at 25° C.; in ether 25 gm. in 100 ml. at 20° C.; in water only 6.9 gm. in 100 ml. at

20° C.), its penetration is not spectacular. Also, mercuric cyanide, in spite of its moderately good lipid-solubility, does not penetrate the skin (94).

Metallic mercury when applied to the skin in ointments penetrates the intact skin through hair follicles and sebaceous glands. Droplets of metallic mercury are found in the appendages and in the upper horny layer but never in the epidermis proper. Calomel (27) and yellow mercuric oxide (60) behave similarly. As mentioned above (p. 29), Moncorps (107) noted traces of mercury in the intracellular spaces of the epidermis after inunction of ammoniated mercury; but he presented evidence that this absorption occurs only if the ammoniated mercury (mercuric amino chloride) is split and mercuric bichloride is formed. Siemens (139) studied this splitting in detail.

Ammoniated mercury itself, which is insoluble in water, in lipids, and in organic solvents, is the least absorbable mercury compound used in therapy. Its bactericidal effect is based on its adsorption to the cell wall and on an extremely slow dissociation of mercuric ions under the influence of the acid reaction of the horny layer and sweat (107, 112). Its extremely low-grade penetration has often been confirmed (123). After percutaneous application of 1 gm. of 10 per cent ammoniated mercury ointment for 4 weeks, Gibbs *et al.* (51) found an average of 24 γ of Hg excreted in the 24-hour urine and only traces in the feces. Robert (119), who applied 10 gm. daily of the same ointment, found between 30 and 95 γ of Hg per liter in the urine and only once as much as 215 γ per liter. According to Robert, these amounts are of no clinical significance. Indeed, it is well known that mercurial poisoning after percutaneous applications of ammoniated mercury ointments is rare. In most instances (119) such cases were apparently due to extreme hypersensitivity to mercury, with traces causing poisoning, or to accidental contamination of the oral cavity (51). Nevertheless, it seems that airtight closure with collodion greatly promotes the decomposition and absorption of

this compound. Grossenbacher (57) found no percutaneous absorption of it at all; but if, under otherwise identical circumstances, the ointment layer was covered with collodion for 27 hours, diarrhea ensued, and mercury could be demonstrated in the urine. One may speculate that retention of sweat promoted formation of mercuric bichloride and that this was absorbed at a higher rate because of aqueous imbibition by the tissues (see p. 52).

In contrast to the poor absorption through intact skin, the absorption of mercury from ammoniated mercury preparations through the mouth (51) and through the vagina (119) is rapid and may be fatal. Remarkably, however, absorption from wound surfaces, particularly from torpid wounds, was found to be not much greater than from normal skin (119). The decisive factor in the case of ammoniated mercury absorption apparently is not the presence or absence of the barrier of normal skin but possibly the pH of the environment. At acid pH's the decomposition of ammoniated mercury is hastened.

The literature on penetration of lead salts through the skin was critically reviewed by Laug (22). Laug and Kunze (81), with great success, used storage in the kidneys (and in other organs) for a measure of percutaneous absorption, a method which served as a "biological magnifier" for absorption of minute quantities. They found much smaller quantities of lead absorbed from lead acetate, lead arsenate, and even from lead oleate than previous authors had. Among the industrially important lead compounds tested, only the volatile lead tetraethyl proved to be absorbed in considerable amount (81). Fatalities from such absorption were reported by Piquet and Hemmeler (113).

Salts of lead, tin, copper, arsenic, bismuth, antimony, and mercury tend to form compounds with the fatty acids of the sebum. Thus an originally lipid-insoluble compound in aqueous solution may be transformed on or in the horny layer into a lipid-soluble metal oleate. The percutaneous ab-

sorption then, quantitatively depends on this transformation. The same reaction may take place outside the body to a larger extent if the heavy metal or its salt is incorporated in ointment bases which contain fatty acids. This is one reason for the increased absorption of heavy-metal salts from some ointments as compared with their absorption from aqueous solutions. This is certainly the case with mercury (83) but, according to Laug and Kunze (81), not necessarily with lead. It appears that the chemical transformation of mercury can be followed even microscopically. While metallic mercury droplets can still be seen in the upper part of the follicle, the rounded, shiny globules tend to become angular and dull particles either in the deeper parts of the follicle or in the sebaceous glands (172, 173, 175).

For absorption of mercury, Laug *et al.* (83) also established the following facts: (1) covering the inunction site increases penetration almost fourfold (see p. 52); (2) there are no regional differences in penetrability of the skin, at least not in the case of laboratory animals; (3) penetration through equal percentages of the total body surface is the same for the rabbit and the rat; (4) if after inunction the excess ointment is removed from the skin surface (as was proposed for practicing "clean inunctions") (27), the absorbed amount is greatly reduced. Halving the exposure areas reduces absorption by one-third.

I. FATTY SUBSTANCES

Animal and vegetable fats and oils enter the pilosebaceous apparatus. The sebaceous-gland cells excrete large fat droplets, and thus it is not surprising to see the reverse process take place, namely, the intake of coarsely dispersed fat. Mineral fats and oils take a similar route. Eller and Wolff (41) confirmed previous data on penetration into sebaceous glands in detailed and careful experiments.

Still, whether these fats are absorbed into the general circulation in any appreciable amounts without being decomposed is high-

ly problematic. Even the amounts which enter the follicles and are imbibed by the horny layer must be relatively small. Bernhardt and Strauch (122), for instance, could recover, without appreciable weight loss, fats which had been applied to the skin for 48 hours. Even if their work was objectionable in some respects (22), it indicates that fatty substances do not enter by the same mechanism and with the same ease as do other "lipid-soluble" substances.

I assumed (122) that absorption of fatty substances is poor because of their colloidal dispersed state; but Laug (22) thought that the important factor is their insolubility in water, a condition incompatible with the modified Overton theory, which requires both lipid- and water-solubility for a substance to be absorbable. Actually, penetration even into the pilosebaceous appendage is slower, the more hydrophobic the fat is: the penetration of petrolatum is always poor as compared with that of triglycerides. Duemling (37) found that fats themselves do not penetrate deep into the follicle but that the addition of wetting agents promotes their descent. However, he did not demonstrate directly the uptake of fatty substances into the general circulation.

The first step in clarifying the controversial question of absorption of fatty material by the radioisotope technic was made by Barail and Pescatore (11). They found that if the U.S.P. cold cream is made up with spermaceti which is labeled with C^{14} , no permeation of this spermaceti can be demonstrated. This is the more remarkable, because spermaceti, consisting mainly of cetyl palmitate, is one of the fatty ointment constituents which are fairly hydrophilic.

J. WATER-SOLUBLE, LIPID-INSOLUBLE NONELECTROLYTES

Theoretically, absorption of such substances is not to be expected through a membrane which is impermeable to liquid water. Experimentally, it has been shown that sucrose and galactose do not penetrate

from ointments, and it is probable that glucose does not either (123). It is regrettable that the available data on this problem are deficient and unsatisfactory.

Immunologic and allergic experiments seem to indicate that lipid-soluble antigenic substances, which usually are large molecules or molecule aggregates, may enter the skin from ointments or wet packs (165, 166, 49, 75). Such results, however, were obtained only after rubbing the antigen ointment vigorously into the skin for prolonged periods of time or on shaved skin. It is doubtful whether in any of these experiments the skin could be regarded as intact. It must also be considered that in the sensitized organism traces of the antigen are sufficient to elicit the immunological reactions which have been used as indicators for the absorption of the antigens. That their absorption is not great in amount is indicated by recommendations to increase it either by previous treatment of the skin with both benzene and petroleum benzene or by electrophoretic introduction (123) or by the use of penetrasols of Herrmann, Sulzberger, and Baer (64).

K. GASES

Substances which are in the gaseous form at ordinary temperatures easily penetrate the skin, with the remarkable exception of carbon monoxide. It is generally assumed that there is a simple diffusion of gases across the entire skin (epidermis and appendages), depending on the difference in concentration or in vapor tension inside and outside the skin and depending on the temperature and on the water- and fat-solubility of the gas (22). Ease of percutaneous absorption has been demonstrated for the following gases: oxygen, nitrogen, helium, carbon dioxide, ammonia vapor, hydrogen sulfide, hydrogen cyanide, vapors of nitrobenzene, dinitrotoluene, and volatile aromatic oils. Absorption of carbon tetrachloride through the skin of monkeys and its rapid elimination through the respiratory

tract were demonstrated by the use of C^{14} -labeled carbon tetrachloride (13).

Physiologically most important, of course, is the movement of oxygen and carbon dioxide through the skin. In the early literature (127) the inward diffusion of oxygen through the skin was regarded as negligible and unimportant compared with the oxygen uptake through the lungs, the factor being 1:160. Goldschmidt *et al.* (55), however, found that, in local asphyxia, oxygen enters the skin from an oxygen-filled plethysmograph and oxygenates the blood in the cutaneous vessels. This is in line with greater penetration if greater concentration difference prevails, and it shows that under abnormal conditions percutaneous oxygen uptake may have biological significance. This view has been strongly supported by newer experiments of Chambers and Goldschmidt (26). They showed that an increased amount of oxygen is frequently taken up by the lungs if the total skin surface is surrounded by nitrogen gas instead of air. The interpretation was that if the body is deprived of a cutaneous oxygen supply, a compensatory mechanism sets in in the respiratory tract. Shaw, Messer, and Weiss (134) found that oxygen is absorbed by human skin even in an atmosphere which contains less than 0.5 per cent oxygen. These authors emphasized that there is never a passage of oxygen outward. Still, the "irreciprocal" permeability of the skin for oxygen has not been directly demonstrated (cf. chap. 24).

The passage of carbon dioxide outward and inward is well established (127, 62). The inward penetration from aqueous solutions is greater than that of the dry gas (56, 133). Hediger (62) found penetration inward at room temperatures as long as the water in his chamber contained more than 4 per cent CO_2 . If the concentration was less, carbon dioxide diffused in the opposite direction until the concentration of 3.8 per cent was reached in the chamber. Kramer and Sarre (77) obtained similar results. Under physiological conditions the carbon dioxide moves steadily outward. The total amount

of CO_2 escaping through the total skin surface, except the head, of an adult man is 7–9 gm. in 24 hours (127). This amount suddenly and abruptly increases when the temperature is raised to the critical temperature of visible sweat secretion (127) (p. 581).

The percutaneous absorption of gases which are foreign to the body has been studied in numerous publications from the toxicological and industrial-hygiene point of view. The capacity of the skin and mucous membranes to absorb hydrogen cyanide gas was dealt with by Flury and Wather (46). When aqueous solutions of hydrogen sulfide are applied to the skin in irritating concentrations, absorption is relatively smaller than in low concentrations (130). Laug and Draize (80) were able to poison rabbits by exposing 9.3 per cent of the body surface to pure hydrogen sulfide.⁵ Experiments with aqueous solutions of hydrogen sulfide at various pH's (120) have revealed that the nondissociated H_2S molecule penetrates better than do the SH^- or NaS^- ions. Ammonium sulfide, however, seems to penetrate better than hydrogen sulfide (80). If elemental sulfur is applied to the skin in ointment vehicles, its absorption occurs in the form of sulfides (104, 106). Cases of poisoning with sulfur ointments were reported as having signs and symptoms of hydrogen sulfide poisoning (sulf-hemoglobin formation) (12). Lietha (87), working with a sulfurated oil ("thiorubrol") containing elemental sulfur, found with Bürgi's method, a decrease in sulfur concentration of the fluid in the bell, which was cemented to the skin, and an increase of the S content in the blood. In one instance he even found the glutathione content of the blood to be increased after percutaneous application of S but felt that this finding still had to be confirmed. The percutaneous absorption of radon was mentioned on page 32. This gas penetrates with greater ease from aqueous solutions than as a dry gas (72).

5. Laug (22) is in error when he believes that I attributed H_2S poisoning in percutaneous experiments to technical imperfections. I never said that.

XI. PRACTICABILITY OF PERCUTANEOUS ADMINISTRATION

The easy penetration of lipid-soluble hormones, vitamins, and pharmacologically active bases revives, time and again, the old problem as to whether percutaneous application of drugs has any advantage over the peroral and/or parenteral ways. Such an advantage is present if one wants to create a slowly but steadily increasing level of the drug in the blood and tissues up to a certain maximum, which may then be kept constant by daily inunctions. In the past the classical mercury inunctions in the treatment of syphilis were advocated mainly on this basis, although similar absorption rates and concentrations have been achieved with intramuscular injections of insoluble salts ("depot effect"). Obviously, such an advantage is not sought in the administration of hormones and vitamins. The aim of such therapy is saturation of the deficient organism. The saturation may be achieved through the administration of the missing factor in any mode of application, and the speed of absorption is of no great importance. If anything, rather rapid absorption is desirable in vitamin and hormonal deficiencies. Lately, greatly prolonged action after percutaneous application (as contrasted with parenteral injections) was observed by Valette and Cavier (158) for estrogenic hormone, and by Bischler *et al.* (14) for oleate of atropine, scopolamine, and hyoscine.

Another reason for percutaneous drug application, however, is to create a higher concentration of the drug in a certain region of the body. It has been claimed that such an effect occurs, e.g., when salicyl ester preparations are rubbed into the skin locally above the painful muscles in myalgia. There is little doubt that introduction of a drug into the skin may temporarily cause a relatively high concentration of the drug in the

tissues close to the site of application, although this never has been conclusively demonstrated in reference to muscles. When in the treatment of deep-seated organs local percutaneous therapy appears to be superior to systemic administration, this superiority results from local vasomotor reflex processes rather than from higher drug concentrations.

In the skin itself a locally higher concentration at the site of application has been unequivocally proved in many instances. A classical example is the unilateral swelling (71) and unilateral hyperpigmentation (32) of the nipples in response to unilateral application of estrogenic hormone. It goes without saying that the principle of topical dermatotherapy as a whole rests on the creation of a locally high concentration of drugs in the skin without appreciable systemic effect. There can be no doubt that this principle can be put into practice. On the other hand, in dealing with lipid-soluble materials one should always be aware of the possibility of massive absorption and consecutive undesirable systemic effects. A particularly dangerous feature of topical applications is that in most cases the absolute amount of the drug placed on the skin is not measured.

Among the widely used drugs which have been reported lately to cause systemic poisoning after percutaneous application are the "cold-wave" materials thioglycerol and thioglycolic acid (36, 162), the antiscabious hexachlorocyclohexane (102), and DDT (24). Recently much attention has been paid to the anticholinesterase poisons, which are all easily absorbed by the skin (102). Absorption of DFP containing radioactive phosphorus has been followed continuously with an elaborate technic by Marrazzi *et al.* (61, 53) in rabbits.

XII. FACTORS PROMOTING PERCUTANEOUS ABSORPTION

In dealing with the factors promoting percutaneous absorption, we distinguish those which promote absorption without

any damaging effect from those which apparently break down the epidermal barrier, whereby nonphysiological conditions are

created. Within the first group two types of promoting factors are conceivable: factors promoting transepidermal, and factors promoting transfollicular, absorption. The only factor which seems to promote transepidermal absorption is moisture.

A. EFFECT OF VEHICLES

Substances soluble in both water and lipids, such as salicylic acid, penetrate with great ease from aqueous solutions as well as from ointments or from fat solvents. The earlier view that, in general, absorption from fats (ointments) and from fat solvents is more rapid and more complete has proved to be not always true. It has been found, for instance, that sex hormones are better absorbed from aqueous vehicles than from oils (42, 109). MacKee *et al.* (93) stated that "various substances can be carried through by means of water," meaning that there can be considerable absorption *from* aqueous solutions.

As pointed out long ago (122), a survey of the available data clearly indicates that the permeation of a substance depends primarily upon its own lipid- and water-solubility. The kind of vehicle in which it is incorporated and the solubility of the substance in the vehicle are of secondary importance. Or, as expressed by Bliss (15): "The properties and powers of the drug itself rather than the ointment vehicle are the major determining factors in absorption from the skin." This does not mean that there are no differences at all in absorption according to the nature of the vehicle. It does mean, however, that, once a substance by virtue of its physical and chemical structure is unable to pass the epidermal barrier, no vehicle will be able to "carry" it through the epidermis. In the case of substances which penetrate it anyhow, the vehicle cannot play a decisive role, because the penetration is rapid and independent of the carrier. Thus the claim, for instance, that one certain oil-in-water emulsion increases the absorption of salicylic acid forty fold as compared with other ointment bases (103, 105) was rather surprising and could not be confirmed (142).

Increase of absorption by choice of a suitable vehicle is always referable to promotion of transfollicular absorption. This point is well illustrated in the case of the absorption-promoting vehicles of MacKee *et al.* (93) (p. 33). None of their vehicles was shown to break the epidermal barrier either from above or from below. On the contrary, when they promoted absorption of substances which otherwise would apparently not enter through the skin or would enter in nondemonstrable traces, the effect was achieved by increased transfollicular absorption. Shelley and Melton (136) confirmed that these bases promote the absorption of water-soluble salts of biologically active bases. They demonstrated absorption of pilocarpine nitrate, acetylcholine chloride, epinephrine hydrochloride, and histamine phosphate by their respective biological action. However, characteristically, the vasoconstrictor effect of the epinephrine salt and the wheal formation in response to the histamine salt were strictly perifollicular. These authors also found that these vehicles do not promote absorption from palms and soles, which have no follicles, and that there is greater absorption from more hairy parts than from parts with less hair. The situation is similar with potassium iodide, which in aqueous solutions penetrates through the follicles only in traces but can be forced through in larger amounts by rubbing it in with ointments (112).

The mechanism whereby a vehicle may promote transfollicular absorption is essentially twofold. The first is an increase in wetting. It is based on diminishing the surface tension between the liquid and the follicular pore and between liquid and air, so that droplet formation is counteracted and a continuous surface layer can be maintained at the liquid/solid boundary. The second is the mechanical factor of bringing the material into more intimate contact with the follicular pore or, more than that, of pressing it into the follicular canal with displacement of air. Thereby incorporated substances are brought more rapidly and in larger amounts to the absorbing surface of

the sebaceous-gland cells and follicular side walls than is possible with other vehicles. The latter effect is best achieved by supple ointments, which can be spread over the skin in a continuous thin layer. If such an ointment is applied with pressure, the gas bubbles adherent to the pores escape sideways; deeper in the follicles and gland ducts they become compressed and absorbed. This, then, is an entirely different situation from that of applications in baths or with wet dressings. If the ointment is tough, tenacious, stiff, or sticky, it cannot be well spread, pressed, or massaged and will not promote absorption (122, 123, 79). The ointment bases themselves penetrate into the follicular openings only, and therefore they can increase the absorption of incorporated substances only via this route. There is no evidence that either ointments or any other kind of "vehicle" may serve as a transportation medium into the cell itself (123, 140). On the contrary, data are available that vehicle and incorporated substance are separated from each other in the follicle and are absorbed at different rates of speed (172, 173).

After attention was directed to the separation of drug and vehicle, the idea arose that it might be advantageous in promoting absorption to choose vehicles which do not bind the incorporated drug too strongly, because the drug has to separate from the base before it enters the cells. It was assumed that the greater the tendency to separation, the more intense is the absorption (123, 153). This idea has held sway up to recent times and seemed to be supported also by clinical experience. However, the principle of easy release cannot be driven too far: if a solid substance is entirely insoluble or remains undispersed in the vehicle—in other words, if it is not "bound" by the vehicle at all—the coarse particles will not penetrate, or their penetration will depend on the particle size. Finely distributed solid material penetrates better than larger particles of the same substance. This was shown for calomel by Laug *et al.* (78). The partial success of the principle of separation of drug from vehicle

certainly militates against the older idea that the drug is carried all the way by the vehicle.

B. OIL-IN-WATER EMULSIONS

Oil-in-water emulsions deserve special mention because of the claim that they would greatly promote percutaneous absorption of incorporated drugs. What actually has been proved beyond doubt concerning these "washable bases" is that denuded and oozing surfaces are much better penetrated by bactericidal compounds from such vehicles than from greasy bases. The cause of the inferior effect of greasy bases is obviously a physical one: the moist surface is not miscible with the grease, and therefore the drug has insufficient contact with the wound surface. One often sees, 24 hours after a greasy ointment layer has been applied to the broken skin with a dressing, that the ointment is completely separated from the moist surface. Eisner (40) showed that layers of oil as thin as 0.07 mm. effectively hinder the penetration of aqueous solutions of electrolytes.

Zheutlin and Fox (174) measured the amount of water-soluble drugs released from various bases into a surrounding aqueous buffer solution resembling extracellular fluid. They found the release of the drugs from water-miscible bases and from oil-in-water emulsions to be 50 to 100 times greater than their release from greasy bases and from water-in-oil emulsions.

Concerning promotion of penetration through intact skin, the washable bases did not fulfil expectations. After great initial enthusiasm it was found that it makes little difference in the rate of absorption whether oil-in-water or water-in-oil emulsions are used as vehicles. This was shown for sulfonamides by Strakosch and Clark (144) and for mercury by Laug *et al.* (82). Of course, negative results indicate only that, in general, no superiority can be attributed to a vehicle according to the type of emulsification. A dispersed aqueous phase in a continuous oily phase, as represented by the traditional cold creams, are not necessarily

inferior to washable bases in which the oily phase is dispersed and the aqueous phase is continuous. However, many of the washable bases contain wetting agents, and mixed with some emulsion bases these agents may have a considerable promoting effect on absorption (37, 82). But even the wetting agents do not always have a demonstrable effect. None was found by Strakosch and Clark (144) for sulfonamides, and none by Shelley and Melton (137) for histamine base and histamine salts. Zopf *et al.* (97), investigating absorption of potassium iodide and of phenolsulfophthalein from various bases by the intact skin of albino rats, found no promoting action of the wetting agent "Tergitol 4." However, they did find definite differences among the vehicles in their promoting effect on absorption of the two compounds tested in the following order: carbowax > hydrophilic ointment U.S.P. > white ointment U.S.P., i.e., a decreasing order with decreasing "washability."

From reviewing the vast literature on the problem of vehicles it becomes evident that different bases are optimal for various substances and that there is not much point in searching for *the* best type of vehicle. There is only one quite general rule, and that is that a vehicle containing fatty substances should be supple.

C. ORGANIC SOLVENTS AND ESSENTIAL OILS

The use of organic solvents, such as ether, chloroform, benzene, petroleum benzine, and, to a lesser degree, alcohol, usually enhances percutaneous absorption. These solvents in themselves increase the permeability of the skin by attacking lipid building stones of the cell membranes (123). If the skin is treated with such solvents in advance, previous to the absorption experiment, increased absorption is subsequently observed from aqueous solutions as well as from any other vehicle. Whether the bursting of the lipid frame concerns epidermal cells or whether the effect may remain restricted to sebaceous-gland cells, so that only transfollicular absorption is promoted,

has not been investigated. Organic solvents may promote absorption to a moderate degree also by dissolving sebum on the skin surface and in the pilosebaceous apparatus and thereby improving conditions for wetting and penetration.

Luduena *et al.* (89) found a pronounced promoting effect of a solvent mixture consisting of diethylene glycol monethyl ether and octyl alcohol on the absorption of mecholyl and epinephrine salts, but not for other drugs. They agree with Rothman and Flesch (126) that "choosing a substance to which the skin is completely impermeable, one will be unable to enforce absorption with any kind of ointment." Miescher (98) noted that absorption of lipid-insoluble materials is not promoted from organic solvents.

Terpenes and terpene derivatives in essential oils have been claimed to promote absorption. Macht (91) found wintergreen oil very effective as a solvent for other lipid substances; and Valette and Valette and Cavier found that water-soluble salts, as well as lipid-soluble substances are better absorbed if dissolved in eucalyptol than from alcoholic solutions (156, 157, 158, 159).

D. PHYSIOLOGICAL STIMULATION

Physiological stimulation of the skin leads to a reversible decrease of the electrical pseudo-resistance, which indicates reversibly increased permeability within the epidermis (p. 21). Actually, increased absorptivity due to physiological stimulation was demonstrated in the frog's skin in a great number of experiments; and there can be little doubt that in lower forms of vertebrates and in invertebrate animals the variability in the permeability of the skin has paramount significance in the percutaneous uptake of food and in the excretion of metabolic end-products. For mammals, however, though the permeability of the epidermis changes on physiologic stimulation, increased absorptivity under the effect of such stimulation has not been demonstrated. Obviously, with the phylogenetic development of the animal kingdom, absorption and

excretion through the skin have gradually lost more and more of their physiological significance (122).

A "stimulation," however, which in its intensity goes beyond the physiological boundary, causing an injury from which the tissue does not recover in a few minutes but the repair of which requires several hours, will measurably increase permeability in mammals also. Under the influence of ultraviolet light, for instance, there is an intravitaly increased diffusion of trypan blue (38), diffusion of calcium salts from connective tissue into the epidermis, and increased penetration of weak alkalies (50). Even in these instances the increased permeability was observed within the skin and not for substances penetrating from the outside. Hitherto the breakage of the epidermal barrier could be demonstrated by absorption experiments only in cases of frank pathological irritation of the skin.

E. ELECTROPHORESIS

"Electrophoresis" was suggested as a term to designate the transport of substances through the skin by direct electric current (122, 123, 76). "Electrophoresis," meaning merely electrical transfer, does not involve any assumption concerning the mechanism of this transport. The expressions "ion transfer" and "iontophoresis" imply that only ions are transferred in one direction or the other, depending on their charges. However, if a galvanic current is sent through the body, in addition to ion transfer we also have to deal with (a) cataphoresis, i.e., transportation of nondissociated molecules and colloids, and (b) electro-osmosis, i.e., shifting of water through a membrane structure with an electrical charge (p. 11).

By applying a galvanic current to the skin, substances can be introduced into the circulation which otherwise would not penetrate or would do so only in traces. This effect is achieved without any demonstrable injury and with as low electric densities as 0.1 M.A./sq cm. The technic of clinical electrophoresis has often been described

(123, 22, 76). The method was rediscovered again and again as a therapeutic tool and was believed to accomplish more than other methods of application. In spite of some interesting results—e.g., mecholyl iontophoresis in peripheral vascular diseases—the therapeutic achievements of electrophoresis have been rather meager. The reason probably is that galvanic current transports drugs quickly into the blood. The only instance in which prolonged remanence in the skin was reported after electrophoresis, as compared with other methods of introduction, is the observation of Abramson and Gorin (4), who found histamine to stay *in situ* for 1 week. This was concluded from the observation that secondary wheals could be produced at the original site of electrolytic histamine introduction 1 week later, when "wet cotton" was placed on the original site and an electric current was sent through. This, of course, does not prove that the originally introduced histamine was still there.

As long as there is no damage to the skin by the current itself, the promotion of absorption by electrophoresis is via the follicles and possibly the sweat-gland ducts. Water-soluble substances are, so to speak, pulled through these appendages, and the rapidity of absorption is often dramatic. This was demonstrated by Lédue in 1900 (84). A rabbit painted with a solution of strychnine nitrate survived this treatment without the slightest signs of poisoning, whereas another rabbit into which the same solution was introduced electrophoretically died in a few minutes from strychnine poisoning. The increase of absorption through appendages is obviously tremendous.

When substances are introduced into the skin electrophoretically, ion transfer, cataphoresis, and electro-osmosis are involved. If the biologically active part of an electrolyte is a cation, the drug is applied at the positive pole (anode), and the cation will travel toward the negative pole, i.e., toward the inside of the body. The inactive anion will travel in the opposite direction, i.e., from the soaked gauze to the metal elec-

trode. Conversely, a substance with an active anion will penetrate to the inside of the body from the negative pole (cathode). Correspondingly, metal ions and organic bases are introduced from the positive pole; iodide and bromide and other anions from the negative pole.

Electro-osmosis and cataphoresis—the transport of the liquid as a whole—interfere with this simple mechanism (117). The skin has a negative charge, and therefore electrophoresis will effect a movement of water into the body from the positive pole and toward the outer surface of the skin at the negative pole. A gross sign of this movement of water is a shrinking of the skin pores at the positive pole and a swelling at the negative after intensive electrophoresis. In case of cation transfer from the positive pole, electro-osmosis acts in the same direction, thus facilitating the absorption of the cation. The anion transfer from the negative pole, however, is counteracted by electro-osmosis, which elicits a movement in the opposite direction. Difficulties encountered in the introduction of dye anions (“acid dyes”) were interpreted as being due to lack of electro-osmosis in the same direction (117).

The role of electro-osmosis was proved by experiments indicating that any factor which furthers electro-osmosis also favors electrophoretic introduction from the positive pole. Such factors are the addition of sucrose or of alcohol to aqueous solutions. These factors are unfavorable for ion transfer but enhance electro-osmosis. Conversely, high salt concentration or acidification has an opposite effect; both are unfavorable for electro-osmosis and hinder permeation through the skin from the positive pole (117). In contrast to cation introduction, the entrance of anions is furthered if electro-osmosis is suppressed by the addition of acids, of multivalent cations, or of concentrated salt solutions. The addition of sucrose or alcohol inhibits the entrance of anions (117).

Rein’s interpretation of the mechanisms regulating electrophoresis was found to be unsatisfactory by Abramson and Gorin (4,

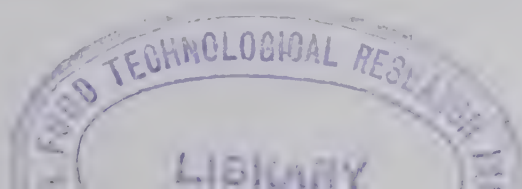
3). These authors found that negative dyes enter with as much ease from the cathode as do positive dyes from the anode. However, they actually did not demonstrate penetration of dyes, only uptake of dyes by the sweat-gland pores (p. 33). They also found that an electrophoretically separated, homogeneous, negatively charged single component of a ragweed allergen could be introduced from the positive pole, and this finding was hard to interpret on the basis of either ion transfer or cataphoresis. Therefore, the authors developed a theory that, in addition to iontophoretic and electro-osmotic velocities, a diffusion velocity factor comes into operation after the electro-osmosis has displaced the air with liquid, so that a “liquid bridge” is in contact with the liquid on the outside of the skin and with the gels and fluids in the skin. Diffusion sets in wherever concentration gradients exist.

Because electrophoresis enables us to introduce substances into the skin without appreciable traumatization and because the dosage can be well standardized, it is a valuable tool for experimental work. Lately it has been extensively used in quantitative experiments on the effects of analgesics, antipruritics, antihistaminics, etc. (115, 135). It has also been recommended for diagnostic purposes, mainly for the introduction of allergens (1, 138).

F. EFFECT OF CONCENTRATION

No increased absorption of sulfonamides can be obtained by increasing the concentration of the compound from 1 to 10 per cent in the vehicle (143). Similarly, it was found that the concentration of mercury in different preparations (82) has very little influence on the rate of percutaneous absorption. The reason probably is that with these nonphysiologic substances, even in the lowest concentrations tested, the difference between outside and inside concentration is large enough to warrant the maximal possible rate of speed of the inward movement.

In contrast to these observations, great differences were found in the whealing reac-



tions to 0.1, 1, 5, and 10 per cent solutions of percutaneously introduced histamine base and histamine salts (137). The cause for the difference might be this: Sulfonamides and mercurials were applied for considerable periods of time with or without dressings, whereas histamine was rubbed in for 1 minute. Histamine is rapidly decomposed within the skin; the other drugs are metabolized slowly. Thus the arrangement with histamine represents an acute experiment in which histamine enters, reacts with the blood vessels, and disappears in an extremely short time, no matter how long the whealing reaction lasts. The strength of the reaction depended on the amount of histamine which the rubbing allowed to enter within 1 minute. Moreover, like any biological reaction, histamine whealing requires a minimum threshold concentration. This is around 1:1,000,000 (see p. 142), and apparently this concentration was not reached at the capillary wall when 0.1 per cent concentrations were applied to the skin in Shelley's experimental arrangement.

G. EFFECT OF MOISTURE

Two opposite views have been prevalent in this problem. From what was said about relative impermeability of the epidermis to water from the outside and about rete cells not being influenced by maceration of the horny layer, one would assume that moisture on the outside does not promote transepidermal absorption. On the other hand, it has been a long-standing clinical experience that watertight covering of the skin surface promotes percutaneous absorption. The keratolytic and acantholytic effects of salicylic acid, for instance, undoubtedly diminish by using vehicles in the order of decreasing occlusive effect, such as plaster > colloidion > ointment > paste > lotion > aqueous and alcoholic solutions. The absorption-promoting effect of occlusion has been attributed to the suppression of insensible water loss and subsequent imbibition of water by the skin, particularly since it was shown that these vehicles decrease insensible water loss in the same order. In

1921 I found that insensible water loss is decreased, on the average, by 50 per cent if ointments, by 25 per cent if pastes, and not at all if dusting powders, are applied to the skin (121). Laug *et al.* (79), too, find that occlusion brings about a more favorable situation for the penetration of some substances.

That moisture plays a decisive role in transepidermal absorption was assumed after it was noted that much more severe lesions develop on human skin if vesicant gases—mustard and nitrogen mustards—are applied to wetted skin than to dry skin, and after this was demonstrated under experimental conditions by Renshaw (118) and by Cullumbine (30). A similar observation was made by Thomson *et al.* (150) for lewisite; they tested the decontaminating effect of different dithiols on the vesicant action of lewisite and found that the protecting effect of BAL is much greater at 63° F. and 37 per cent humidity than at 86° F. and 50 per cent humidity.

Whereas lewisite penetration might be transfollicular, mustard and nitrogen mustards certainly penetrate directly into the epidermis (p. 39). This promotion of absorption by moisture remains unexplained. It is known, however, that, in contact with water, mustards transform to various highly soluble, reactive, and toxic intermediary products before the inactive end-products of the water-mustard reactions are formed (52, 7). Thus it is feasible that more intense reactions in the presence of moisture are not due to increased absorption but to chemical changes of the material. Judgment should be withheld until it is shown that substances which do not react with water are shown to penetrate the epidermis better in the presence of moisture than through a dry skin surface.

Evidence concerning the promoting effect of moisture on transfollicular absorption was presented by Laug *et al.* (83). They demonstrated that the absorption of mercury is increased nearly fourfold by covering the site of inunction. The authors assume that the cover acts by interfering with the

normal escape of moisture: moisture produces softening and maceration of the horny layer and thus creates a condition favorable for retention of substances in close contact with the skin (86). Possibly an observation

of Shelley and Melton (137) also belongs here. They found a prolonged effect of histamine when the solution applied to the skin was covered so that evaporation of water was inhibited.

BIBLIOGRAPHY

1. ABRAMSON, H. A. Skin reactions. X. Pre-seasonal treatment of hay fever by electrophoresis of ragweed pollen extracts into the skin. Preliminary report, *J. Allergy*, **12**:169-75, 1941.
2. ABRAMSON, H. A., and ENGLE, M. C. Skin reactions. XII. Patterns produced in the skin by electrophoresis of dyes, *Arch. Dermat. & Syph.*, **44**:190-200, 1951.
3. ABRAMSON, H. A., and GORIN, M. H. Skin permeability, Cold Spring Harbor Symp. Quant. Biol., **8**:272-79, 1940.
4. ———. Skin reactions. IX. The electrophoretic demonstration of the patent pores of the living human skin: its relation to the charge of the skin, *J. Phys. Chem.*, **44**:1094-1102, 1940.
5. ALBRIEUX, A. S.; POSE, G.; and PREGO, L. E. Acción de una preparación de tiroides a traves de la piel del cobayo, *Arch. clin. e inst. de endocrinol.*, **3**:70-72, 1943-46.
6. ANONYMOUS. Government and industry cooperate on tetraethyl pyrophosphate report, *Chem. Eng. News*, **26**:2356, 1948.
7. ANSLOW, W. P., JR.; KARNOFSKY, D. A.; JAGER, B. V.; and SMITH, H. W. Intravenous, subcutaneous and cutaneous toxicity of bis(β -chlorethyl)sulfide (mustard gas) and of various derivatives, *J. Pharmacol. & Exper. Therap.*, **93**:1-9, 1948.
8. ANTINOBIN, A. Ricerche quantitative sull' adsorbimento cutaneo, *Arch. internat. de pharmacodyn. et de therap.*, **31**:351-57, 1926.
9. AXELROD, D. J., and HAMILTON, J. G. Radio-autographic studies of the distribution of lewisite and mustard gas in skin and eye tissues, *Am. J. Path.*, **23**:389-411, 1947.
10. BEHNKE, A. R., and WILLMON, T. L. Cutaneous diffusion of helium in relation to peripheral blood flow and absorption of atmospheric nitrogen through skin, *Am. J. Physiol.*, **131**:627-32, 1941.
11. BARAIL, L. C., and PESCATORE, J. J. Radioactive carbon shows spermaceti in cold cream doesn't penetrate skin, *Drug Trade News*, Vol. **41**, December 12, 1949. Quoted by Nuclear Sc. Abstr., Vol. **4**, No. 1370, 1950.
12. BASCH, F. Über Schwefelwasserstoffvergiftung bei äusserlicher Applikation von elementarem Schwefel in Salbenform, *Arch. f. exper. Path. u. Pharmacol.*, **111**:126-32, 1926.
13. BEAMER, W. H.; MCCOLLISTER, D. D.; ATCHISON, G. J.; and SPENCER, H. C. Studies with low vapor concentrations of carbon tetrachloride labeled with C¹⁴. II. Absorption with elimination upon skin exposure of monkeys, *Federation Proc.*, **9**:257, 1950.
14. BISCHLER, A.; FAVRE, M.; FROMMEL, E.; and VALETTE, F. Permeability of the guinea pig's skin to oleates of the alkaloids of poisonous solanaceae measured by acetylcholine aerosols, *Acta pharmacol. et toxicol.*, **4**:122-26, 1948.
15. BLISS, A. R. The absorption of certain drugs from the human skin, *J. Am. Pharm. A. (scient. ed.)*, **25**:694-701, 1936.
16. BROWN, E. V., and SCOTT, W. O. The absorption of methyl salicylate by the human skin, *J. Pharmacol. & Exper. Therap.*, **50**:32-50, 373-85, 1934.
17. WINSOR, T., and BURCH, G. E. Differential roles of human epigastric skin on diffusion rate of water, *Arch. Int. Med.*, **74**:428-36, 1944.
18. BURCH, G. E., and WINSOR, T. Diffusion of water through dead plantar, palmar, and dorsal human skin and through toe nails, *Arch. Dermat. & Syph.*, **53**:39-41, 1946.
19. BÜRGI, E. La perméabilité de la peau au mercure, *Rev. méd. de la Suisse Rom.*, **57**:461, 1927. Quoted by BÜRGI (20).
20. ———. Die Durchlässigkeit der Haut für Arzneien und Gifte. Berlin: J. Springer, 1942.
21. BURLET, E. Die perkutane Resorption von Panthenol. Jubilee vol. for Emil Barell,

- pp. 92-97, 1946. Quoted by COMBES, F. C., and ZUCKERMAN, R., *J. Invest. Dermat.*, **16**:379-81, 1951.
22. CALVERY, H. O.; DRAIZE, J. H.; and LAUG, E. P. The metabolism and permeability of normal skin, *Physiol. Rev.*, **26**:495-540, 1946. Section on permeability by LAUG, E. P., pp. 510-33.
 23. CALVERY, H. O.; DRAIZE, J. H.; and WOODARD, G. Relative acute toxicity of some organic compounds following oral administration and skin application, *Fed. Proc.*, **3**:67, 1944.
 24. CASE, R. A. M. Toxic effects of 2,2-bis(*p*-chlorophenyl)1,1,1-trichlorethane (DDT) in man, *Brit. M. J.*, **2**:842-45, 1945.
 25. CASTOR, C. W., and BAKER, B. L. Local action of adrenocortical steroids on epidermis and connective tissue of skin, *Endocrinology*, **47**:234-41, 1950.
 26. CHAMBERS, A. H., and GOLDSCHMIDT, S. The influence of cutaneous atmospheric oxygen absorption upon the apparent total oxygen utilization of the body, *Am. J. Physiol.*, **129**:331-32, 1940.
 27. COLE, H. N.; DE WOLF, H. F.; SCHREIBER, N. E.; SOLLMANN, T.; and VAN CLEVE, J. Mercurial inunctions in the treatment of syphilis: excretion of mercury following use of mild mercurous chloride inunctions; mode of absorption of mercury from skin, *Arch. Dermat. & Syph.*, **27**:1-11, 1933.
 28. CRONIN, T. D., and BAUER, R. O. Death due to phenol contained in foille, *J.A.M.A.*, **139**:777-79, 1949.
 29. CULLUMBINE, H. The mode of penetration of the skin by mustard gas, *Brit. J. Dermat.*, **58**:291-94, 1946.
 30. ———. Factors influencing penetration of the skin by chemical agents, *Quart. J. Exper. Physiol.*, **34**:83-89, 1948.
 31. CUTNER, M. The influence of "red light" and of ionization upon the penetration of the skin by penicillin, *Brit. J. Phys. Med.*, **12**:144-47, 1949.
 32. DAVIS, M. E.; BOYNTON, M. W.; FERGUSON, S.; and ROTHMAN, S. Studies on pigmentation of estrogenic origin, *J. Clin. Endocrinol.*, **5**:138-46, 1945.
 33. DEICHMANN, W. B. Local and systemic effects following skin contact with phenol—a review of literature, *J. Indust. Hyg. & Toxicol.*, **31**:146-54, 1949.
 34. DEICHMANN, W. B.; MILLER, T.; and ROBERTS, J. B. Local and systemic effects following application of dilute solutions of phenol in water and in camphor-liquid petrolatum on the skin of animals, *Arch. Indust. Hyg.*, **2**:454-61, 1950.
 35. DEICHMANN, W. B.; RUEDEMANN, R.; and MILLER, T. Absorption of phenol following application of 2% phenol in calamine and "campho-phenique" to skin of experimental subjects, *Federation Proc.*, **9**:30, 1950.
 36. DRAIZE, J. H.; ALVAREZ, E.; and WOODARD, M. Comparative percutaneous toxicity of 3-mercapto-1,2-propanediol (thioglycerol) and ammonium thioglycolate, *Federation Proc.*, **8**:287, 1949.
 37. DUEMLING, W. W. Wetting agents: new synthetic chemicals of use in finer and more efficient topical dermatologic therapy, *Arch. Dermat. & Syph.*, **43**:264-80, 1941.
 38. ECKSTEIN, A., and MOELLENDORFF, W. von. Histophysiologische Untersuchungen, über den Einfluss der Bestrahlung mit der Quecksilberquarzlampe, *Arch. f. Kinderheilk.*, **72**:205-18, 1923.
 39. EHRHARDT, K. Ueber das Laktationshormon des Hypophysenvorderlappens, *München. med. Wchnschr.*, **83**:1163-64, 1936.
 40. EISNER, H. A method for study of penetrability of liquid and semisolid films used in skin protection, *J. Invest. Dermat.*, **10**:273-79, 1948.
 41. ELLER, J. J., and WOLFF, S. Permeability and absorptivity of the skin, *Arch. Dermat. & Syph.*, **40**:900-923, 1939.
 42. ———. Hormones and vitamins in cosmetics, *J.A.M.A.*, **114**:1865-75 and 2002-10, 1940.
 43. ELLIOTT, J. A., JR., and ODEL, H. M. Percutaneous absorption of sodium in cases of exfoliative dermatitis, *J. Invest. Dermat.*, **15**:389-95, 1950.
 44. FERGUSON, R. L., and SILVER, S. D. Demonstration in skin of active chlorine from chlorine-liberating ointments, *Am. J. Clin. Path.*, **17**:35-36, 1947.
 45. FLESCH, P.; GOLDSTONE, S. B.; and URBACH, F. Palmar pore patterns: their significance in the absorption of dyes, *Arch. Dermat. & Syph.*, **63**:228-31, 1951.
 46. FLURY, F., and WATHER, R. Blausäurevergiftung durch Hautresorption, *Arch. f. Gewerbepath. u. Gewerbehyg.*, **11**: 311-25, 1942.
 47. FREEMAN, M. V.; ALVAREZ, E.; and

- DRAIZE, J. H. Cutaneous absorption of phenol from intact and damaged skin, *Federation Proc.*, **9**:273, 1950.
48. FUHRMAN, F. A., and USSING, H. H. Characteristic response of isolated frog skin potential to neurohypophyseal principles and its relation to transport of sodium and water, *Fed. Proc.*, **9**:46, 1950.
49. GOLOVANOFF, M. De la sensibilisation par la voie cutanée, *Compt. rend. Soc. de biol.*, **94**:6, 1926.
50. GANS, O., and SCHLOSSMANN, H. Über den Einfluss kurzwelliger (ultravioletter) Strahlen auf die Permeabilität der Haut (zugleich ein Beitrag zur Genese der Calciumverschiebung), *Dermat. Wchnschr.*, **80**:469-75, 1925.
51. GIBBS, O. S.; SHANK, R.; POND, H.; and HANSMANN, G. H. Absorption of externally applied ammoniated mercury, *Arch. Dermat. & Syph.*, **44**:862-72, 1941.
52. GILMAN, A., and PHILLIPS, F. S. The biologic actions and therapeutic applications of the β -chlorethyl amines and sulfides, *Science*, **103**:409-36, 1946.
53. GITES, H. R.; HART, E. R.; and MARRAZZI, A. S. Measurement of skin penetration by use of subcutaneous probe-counter in conjunction with radioisotope labelled material, *Federation Proc.*, **10**:50, 1951.
54. GOLDMAN, L.; THOMPSON, R. G.; and TRICE, E. R. Cortisone acetate in skin disease: local effect in skin from topical application and local injection, *Arch. Dermat. & Syph.*, **65**:177-86, 1952.
55. GOLDSCHMIDT, S.; MCGLONE, B.; and DONAL, J. S. Oxygen absorption through the skin, *Am. J. M. Sc.*, **187**:586, 1934.
56. GROEDEL, F. M., and WACHTER, R. Über den Gasstoffwechsel in Süßwasser-, Salz-, Luft- und kohlensaurem Wasser und Gasbad, *Veröffentl. d. Zentralstelle f. Balneol.*, N.F., No. 16, 1929.
57. GROSSENBACHER, H. Über die Penetrationsfähigkeit der weissen Quecksilbersalbe durch die Haut, *Inaug. diss.*, Bern, 1936.
58. GÜNTHER, G. Experimentelle Studien über die Hautresorption, *Prag. Arch. f. Tiermed. u. vergl. Path.*, **6**:55-60, 1926.
59. HALPERN, B. N.; GAUDIN, O.; and STIFFEL, C. Étude de l'influence des cations sur la perméabilité cutanée et cellulaire vis-à-vis de certains dérivés salicylés: recherches sur l'absorption cutanée chez l'homme et l'animal, *Compt. rend. Soc. de biol.*, **142**:819-21, 1948.
60. HARRY, R. G. Skin penetration, *Brit. J. Dermat.*, **53**:65-82, 1941.
61. HART, E. R.; FLEISCHER, J. H.; and MARRAZZI, A. S. Skin penetration of radioactive anticholinesterases—a direct quantitative method of study, *Federation Proc.*, **8**:300, 1949.
62. HEDIGER, S. Experimentelle Untersuchungen über die Resorption der Kohlensäure durch die Haut, *Klin. Wchnschr.*, *Proc.*, **7**:1553-57, 1928.
63. HERRMANN, F. Introduction of allergens into the skin by inunction with "Intra-derm." I. Additional experimental data on the inunction test, *Ann. Allergy*, **3**:431-34, 1945.
64. HERRMANN, F.; SULZBERGER, M. B.; and BAER, R. L. Penetration of allergens into human skin, *New York State J. Med.*, **44**:2452-56, 1944.
65. HOPMANN, F. Über den Einfluss des Kohlensäurebads auf die Permeabilität der Haut für Salicylsäure, *Balneologie*, **6**:5-8, 1939.
66. HUF, E. G., and PARRISH, J. Nature of the electrolyte pump in surviving frog skin, *Am. J. Physiol.*, **164**:428-36, 1951.
67. HUF, E. G.; PARRISH, J.; and WEATHERFORD, C. Active salt and water uptake by isolated frog skin, *Am. J. Physiol.*, **164**:137-42, 1951.
68. ICHIHASHI, T. Effect of drugs on the sweat glands by cataphoresis and an effective method for suppression of local sweating. II. The paths of cataphoresis in the skin and the influence of perspiratory conditions on the cataphoretic effect, *J. Orient. Med.*, **25**:103-4, 1936.
69. ISLER, H. Études sur la résorption cutanée, *Dermatologica*, **100**:295-300, 1950.
70. JACOBS, M. H. Permeability of the cell to diffusing substances. *In*: COWDRY, General pathology, Sec. III, pp. 99, 1924. Quoted by ROTHMAN (123).
71. JADASSOHN, W.; UEHLINGER, E.; and MARGOT, A. The nipple test: studies in the local and systemic effects on topical application of various sex hormones, *J. Invest. Dermat.*, **1**:31-43, 1938.
72. JANITZKY, A.; RASCHIG, W.; STEINKE, O.; and WICHMAN, W. Untersuchungen über die Frage, ob Radiumemanation durch die

- Haut des menschlichen Körpers hindurchgeht, *Klin. Wchnschr.*, **12**:1692-93, 1933.
73. JOHNSTON, G. W., and LEE, C. O. A radioactive method of testing absorption from ointment bases, *J. Am. Pharm. A. (scient. ed.)*, **32**:278-80, 1943.
 74. KAHLENBERG, L. On the passage of boric acid through the skin by osmosis, *J. Biol. Chem.*, **62**:149-56, 1924.
 75. KIMURA, G. Studies on absorbing coloring matter as well as heterogeneous protein through skin at time of unbalanced acid-base equilibrium; examination of skin's function of absorbing coloring matter, *Orient. J. Dis. Infants*, **28**:15, 1940.
 76. KOVACS, R. *Electrotherapy and light therapy*. Philadelphia: Lea & Febiger, 1946.
 77. KRAMER, K., and SARRE, H. Untersuchungen über die Kohlensäurediffusion durch die Haut, *Arch. f. exper. Path. u. Pharmacol.*, **180**:545-66, 1936.
 78. LAUG, E. P. Studies on the permeability of the skin to mercury, *J. Soc. Cosmet. Chemists*, **1**:81-94, 1948.
 79. LAUG, E. P.; AUGHEY, E.; and UMBERGER, E. J. Factors influencing the absorption of mercury from calomel ointments applied to the skin, *Federation Proc.*, **3**:78, 1944.
 80. LAUG, E. P., and DRAIZE, J. H. The percutaneous absorption of ammonium hydrogen sulfide and hydrogen sulfide, *J. Pharmacol. & Exper. Therap.*, **76**:179-88, 1942.
 81. LAUG, E. P., and KUNZE, F. M. Penetration of lead, *J. Indust. Hyg. & Toxicol.*, **30**:256-59, 1948.
 82. LAUG, E. P.; VOS, E. A.; KUNZE, F. M.; and UMBERGER, E. J. A study of certain factors governing the penetration of mercury through the skin of the rat and the rabbit, *J. Pharmacol. & Exper. Therap.*, **89**:52-63, 1947.
 83. LAUG, E. P.; VOS, E. A.; UMBERGER, E. J.; and KUNZE, F. M. A method for the determination of cutaneous penetration of mercury, *J. Pharmacol. & Exper. Therap.*, **89**:42-51, 1947.
 84. LÉDUC, S. *Ann. d'électrobiol.*, **3**:545, 1900. Quoted by ROTHMAN (122).
 85. LEHMANN, G. Über die Resorption von Kohlensäure aus Salzlösungen und von Salzlösungen selbst durch die Haut, *Arch. internat. de pharmacodyn. et de therap.*, **55**:331-46, 1937.
 86. LESLIE-ROBERTS, H. On the absorption of salicylic acid by the human skin, *Brit. J. Dermat.*, **40**:325-44, 1928.
 87. LIETHA, P. Die Resorption von Schwefel aus Thiorubrol durch die Haut, *Schweiz. med. Wchnschr.*, **72**:650-52, 1942.
 88. LOEFFLER, R. K., and THOMAS, V. A quantitative study of percutaneous absorption. I. Absorption of radioactive strontium chloride in minute quantities through intact and mechanically damaged rat skin, *U.S. Atomic Energy Commission Rept. AD-225B; Nuclear Sc. Abstr.*, Vol. **5**, No. 323, 1951.
 89. LUDUENA, F. P.; FELLOWS, J. K.; and DRIVER, R. L. Epidermal absorption of drugs, *Arch. Dermat. & Syph.*, **57**:210-18, 1948.
 90. LUX, R. E., and CHRISTIAN, J. E. Permeability of frog skin by means of radioactive tracers, *Am. J. Physiol.*, **162**:193-97, 1950.
 91. MACHT, D. I. The absorption of drugs and poisons through the skin and mucous membranes, *Arch. internat. de pharmacodyn. et de therap.*, **58**:1-26, 1938.
 92. MCCLELLAN, W. S., and COMSTOCK, C. R., JR. The cutaneous absorption of radon from naturally carbonated water baths, *Arch. Phys. Med.*, **30**:29-36, 1949.
 93. MACKEE, G. M.; SULZBERGER, M. B.; HERRMANN, F.; and BAER, R. L. Histologic studies on percutaneous penetration, with special reference to the effect of vehicles, *J. Invest. Dermat.*, **6**:43-61, 1945.
 94. McNALLY, W. D., and FOSTVEDT, G. Non-absorption of mercuric cyanide from soap, *Indust. Med.*, **13**:543-45, 1944.
 95. MATSCHAK, H. Zur Physiologie und Pharmakologie der Sterine. III. Der Einfluss der Sterine in Salbengrundlagen auf die Resorption percutan verabreichter Arzneimittel, *Arch. f. exper. Path. u. Pharmacol.*, **182**:688-99, 1936.
 96. MEYENBERG. Über das Einverleibungsvermögen (Resorptionsvermögen) der Haut, *Dermat. Wchnschr.*, **112**:31-33, 1941.
 97. MEYERS, D. S.; NADKARNI, M. V.; and ZOPF, L. C. The absorption and penetration of therapeutic agents from a carbowax vehicle, *J. Am. Pharm. A. (scient. ed.)*, **38**:231-34, 1949.
 98. MIESCHER, G. Fluoreszenzmikroskopische Untersuchungen zur Frage der Penetration

- von fluoreszierenden Stoffen in die Haut, *Dermatologica*, **83**:50-62, 1941.
99. MILBRADT, W. Experimentelle Untersuchungen zur Herabsetzung und Förderung der Hautresorption, *Dermat. Ztschr.*, **66**:37-54, 1933.
 100. ———. Der Einfluss der Saponine auf die Durchgängigkeit der Haut, *Ztschr. f. d. ges. exper. Med.*, **87**:745-54, 1933.
 101. MILLER, O. B., and SELLE, W. A. Studies in percutaneous absorption of radioiodine, *J. Invest. Dermat.*, **12**:19-29, 1949.
 102. MOBBS, R. F. Toxicity of hexachlorocyclohexane in scabies: letter to the editor, *J.A.M.A.*, **138**:1253, 1948.
 103. MONCORPS, C. Untersuchungen über die Pharmakologie und Pharmakodynamik von Salben und salbeninkorporierten Medikamenten. II. Über die Resorption und Pharmakodynamik der salbeninkorporierten Salicylsäure, *Arch. f. exper. Path. u. Pharmacol.*, **141**:50-66, 1929.
 104. ———. Untersuchungen über die Pharmakologie und Pharmakodynamik von Salben und salbeninkorporierten Medikamenten. III. Untersuchungen über die Resorption und Pharmakodynamik des salbeninkorporierten elementaren Schwefels, *ibid.*, pp. 67-86.
 105. ———. Untersuchungen über die Pharmakologie und Pharmakodynamik der Salben und salbeninkorporierten Medikamenten. IV. Über die Beeinflussung des Schwefelhaushaltes beim Menschen nach Schwefelsalbenanwendung, *ibid.*, pp. 87-104.
 106. ———. Untersuchungen über die Pharmakologie und Pharmakodynamik der Salben und salbeninkorporierten Medikamenten. V. Über den Sulfatgehalt und Transmineralisationsvorgänge im Blut beim Menschen nach Schwefelsalbenanwendung, *ibid.*, **152**:57-67, 1930.
 107. ———. Untersuchungen über die Pharmakologie und Pharmakodynamik der Salben und salbeninkorporierten Medikamenten. VI. Über die Pharmakologie und Pharmakodynamik des Ungt. Hydr. praec. alb. Ph. G., *ibid.*, **155**:51-69, 1930.
 108. NATHANSOHN, A. Über die Regulation der Aufnahme anorganischer Salze durch die Knollen von Dahlia, *Jahrb. wiss. Bot.*, **39**:607-44, 1904.
 109. NELSON, D.; GREENE, R. R.; and WELLS, J. A. Variations in the effectiveness of percutaneously applied androgens in the rat, *Endocrinology*, **26**:651-55, 1940.
 110. OPPENHEIM, M. Beiträge zur Frage der Hautabsorption mit besonderer Berücksichtigung der erkrankten Haut, *Arch. f. Dermat. u. Syph.*, **93**:85-106, 1908.
 111. OVERTON, E. Studien über die Narkose. Jena: G. Fischer, 1924.
 112. PERUTZ, A. Die Pharmakologie der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **5**:2-249, 1930.
 113. PIQUET, J. C., and HEMMELER, G. Poisoning with gasoline containing tetraethyl lead, *Ärzt. Monatsh.*, **4**:181-90, 1948. Quoted in *Chem. Abstr.*, **42**:5563/b, 1948.
 114. RABEAU, H., and MALANGEAU, P. Actions de certains agents chimiques sur la pénétration cutanée, *Bull. Soc. franç. de dermat. et syph.*, **54**:385-87, 1947.
 115. RASMUSSEN, K. A. The effect of antihistaminics on histamine whealing and on dermographism—elucidated by comparative electrophoretical experiments, *Acta dermat.-venereol.*, **29**:564-71, 1949.
 116. REIN, H. Zur Elektrophysiologie der menschlichen Haut. I. Untersuchungen über die Farbstoffeinwanderung in lebende Warmblüterhaut im elektrischen Felde, *Ztschr. f. Biol.*, **84**:41-50, 1926.
 117. ———. Die Elektrophysiologie der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1**:43-91, 1929.
 118. RENSHAW, B. Observations on the role of water in the susceptibility of human skin to injury by vesicant vapors, *J. Invest. Dermat.*, **9**:75-85, 1947.
 119. ROBERT, P. Klinische Untersuchungen über die Resorption der weissen Präzipitatsalbe durch die intakte Haut und ulzeröse Wundflächen, *Dermatologica*, **92**:85-107, 1946.
 120. ROQUES, E.; CAUJOLLE, F.; and REY, J. La perméabilité cutanée à l'hyposulfite de sodium, à l'hydrogène sulfuré et aux sulfures de sodium, *Bull. Acad. de méd., Paris*, **119**:515-18, 1939.
 121. ROTHMAN, S. Über den Einfluss einiger dermatotherapeutischer Grundsubstanzen auf die insensible Wasserabgabe der Haut, *Arch. f. Dermat. u. Syph.*, **131**:549-65, 1921.
 122. ———. Resorption durch die Haut. In: BETHE, Handb. d. norm. u. path. Physiol., **4**:107-51, 1929, and **18**:85-91, 1932.
 123. ———. The principles of percutaneous

- absorption, *J. Lab. & Clin. Med.*, **28**:1305-21, 1943.
124. ROTHMAN, S. Discussion on: PECK, S. M.; FINKLER, B.; MAYER, G. G.; and MICHELFELDER, T., Effects of various modes of administration of pyribenzamine on the histamine wheal and epidermal sensitivity reactions, *J. Invest. Dermat.*, **14**:177-91, 1950.
 125. ———. Symposium on cortisone and compound F of the College of Medicine, University of Cincinnati, May 1, 1952. Unpublished.
 126. ROTHMAN, S., and FLESCH, P. The physiology of the skin, *Ann. Rev. Physiol.*, **6**:195-224, 1944.
 127. ROTHMAN, S., and SCHAAF, F. Chemie der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1**:161-377, 1929.
 128. SAWYER, W. H. Effect of posterior pituitary extract on permeability of frog skin to water, *Am. J. Physiol.*, **164**:44-48, 1951.
 129. SCHMID, G. Über die Resorption von Kohlensäure und Schwefelwasserstoff durch die Haut, *Arch. internat. de pharmacodyn. et de therap.*, **55**:318-30, 1937.
 130. SCHWENKENBECHER, A. Das Absorptionsvermögen der Haut, *Arch. Anat. u. Physiol.*, pp. 121-65, 1904.
 131. ———. Die Haut als Exkretionsorgan. In: BETHE, Handb. d. norm. u. path. Physiol., **4**:709-68, 1929.
 132. SELLE, W. A., and MILLER, O. B. Fatal gastric secretion of radioiodine applied percutaneously to pregnant animals, *Fed. Proc.*, **7**:110, 1948.
 133. SHAW, L. A., and MESSER, A. C. Cutaneous respiration in man. II. The effect of temperature and of relative humidity upon the rate of carbon dioxide elimination and oxygen absorption, *Am. J. Physiol.*, **95**:13-19, 1930.
 134. SHAW, L. A.; MESSER, A. C.; and WEISS, S. Cutaneous respiration in man. I. Factors affecting the rate of carbon dioxide elimination and oxygen absorption, *Am. J. Physiol.*, **90**:107-18, 1929.
 135. SHELLEY, W. B.; MCCONAHY, J. C.; and HESBACHER, E. N. Effectiveness of antihistaminic compounds introduced into normal human skin by iontophoresis, *J. Invest. Dermat.*, **15**:343-44, 1950.
 136. SHELLEY, W. B., and MELTON, F. M. Studies on absorption through normal human skin, *Federation Proc.*, **6**:199-200, 1947.
 137. ———. Factors accelerating the penetration of histamine through normal intact human skin, *J. Invest. Dermat.*, **13**:61-71, 1949.
 138. SHILKRET, H. H. Electrophoretic skin studies. I. Reaction to common grasses, *J. Invest. Dermat.*, **5**:11-14, 1942.
 139. SIEMENS, H. W., and SCHREIBER, E. Chemische und klinisch-experimentelle Untersuchungen über die Kombination von Quecksilberpräzipität mit Salizylsäure bei der Behandlung der Hautkrankheiten, *Dermatologica*, **93**:1-14, 1946.
 140. STARKENSTEIN, E., and HENDRYCH, F. Zur Physiologie und Pharmakologie der Sterine. II. Die Bedeutung des Cholesterins für Permeabilität und Resorption, *Arch. f. exper. Path. u. Pharmacol.*, **182**:664-87, 1936.
 141. STEIN, I. D., and WEINSTEIN, I. The value of carbon dioxide baths in treatment of peripheral vascular disease and allied conditions, *Am. Heart J.*, **23**:349-61, 1942.
 142. STRAKOSCH, E. A. Studies on ointments. II. Ointments containing salicylic acid, *Arch. Dermat. & Syph.*, **47**:16-26, 1943.
 143. STRAKOSCH, E. A., and CLARK, W. G. Studies on the penetration of sulfonamides into the skin. I. Penetration of sulfonamides from various ointment bases into the intact skin of guinea pigs; and a new method of analysis of tissue sulfonamides, *Am. J. M. Sc.*, **205**:518-24, 1943.
 144. ———. Studies on the penetration of sulfonamides into the skin. II. Sulfathiazole, sulfadiazine and sodium sulfacetamide, *ibid.*, **206**:610-18, 1943.
 145. STROHL, A.; VERNE, J.; ROUCAYROL, J. C.; and CECCALDI, P. F. Étude de l'introduction électrolytique des ions à l'aide d'isotopes radioactifs, *Compt. rend. Soc. de biol.*, **144**:819-24, 1950.
 146. SULZBERGER. In: MACKEE, G. M.; HERRMANN, F.; BAER, R. L.; and SULZBERGER, M. B. Demonstrating the presence of sulfonamides in the tissues, *Science*, **98**:66-69, 1943.
 147. SULZBERGER, M. B., and BAER, R. L. Present status of ACTH, cortisone and compound F in dermatologic management: a guide for the general practitioner. In: Year book of dermatology and syphilology

- 1952, pp. 7-21. Chicago: Year Book Publishers, 1953.
148. SULZBERGER, M. B.; WITTEN, V. H.; and SMITH, C. C. Hydrocortisone (compound F) acetate ointment in dermatological therapy, *J.A.M.A.*, **151**:468-72, 1953.
149. SZCZESNIAK, A. S.; SHERMAN, H.; and HARRIS, R. S. The percutaneous absorption of water, *Science*, **113**:293-94, 1951.
150. THOMSON, J. F.; SAVIT, J.; and GOLDWASSER, E. Tests of 2,3-dimercaptopropanol and related dithiols for decontamination of lewisite on human skin, *J. Pharmacol. & Exper. Therap.*, **89**:1-13, 1947.
151. TRAUBE-MENGARINI, M. Über die Permeabilität der Haut, *Arch. f. Anat. u. Physiol.*, 1892, suppl., pp. 1-10.
152. UNNA, P. G., and SCHUMACHER, J. *Lebensvorgänge in der Haut der Menschen und der Tiere*. Leipzig and Vienna: F. Deuticke, 1925.
153. UNNA, P., JR. Zur Frage der Salbengrundlagen, *Deutsche med. Wchnschr.*, **52**:197-99, 1926.
154. USSING, H. H. Active ion transport through isolated frog skin in the light of tracer studies, *Acta physiol. Scandinav.*, **17**:1-37, 1949.
155. ———. Distinction by means of tracers between active transport and diffusion. The transfer of iodide across the isolated frog skin, *ibid.*, **19**:43-56, 1949.
156. VALETTE, G. Sur la pénétration transcutanée des huiles essentielles et de leurs constituants chimiques, *Compt. rend. Soc. de biol.*, **139**:904-6, 1945.
157. ———. Sur la pénétration transcutanée des alcaloïdes, *ibid.*, pp. 906-7.
158. VALETTE, G., and CAVIER, R. Sur l'activité de la dihydrofolliculine par voie transcutanée chez le rat, *Compt. rend. Soc. de biol.*, **140**:255-56, 1946.
159. ———. Sur l'activité de la testostérone par voie transcutanée chez le rat, *ibid.*, pp. 825-27.
160. ———. Sur l'activité de la désoxycorticostérone par voie transcutanée chez le rat, *ibid.*, **141**:465-68, 1947.
161. ———. L'absorption cutanée, *J. de physiol.*, **39**:137-74, 1947.
162. VAN DER BURG, A. P. Toxicity of "cold wave" solution, *Nederl. tijdschr. v. geneesk.*, **93**:3400-3403, 1949. Quoted in *J.A.M.A.*, **142**:852, 1950.
163. VOSS, H. E. Die örtliche Wirkung von Sexualhormonen, *Klin. Wchnschr.*, **16**:769-71, 1937.
164. WALKER, S. A., and ROTHMAN, S. Alopecia areata: statistical study and consideration of endocrine influences, *J. Invest. Dermat.*, **14**:403-13, 1950.
165. WALZER, A. Cutaneous absorption. I. A direct technic for demonstrating the percutaneous absorption of antigens, *Arch. Dermat. & Syph.*, **41**:692-98, 1940.
166. WALZER, A., and SACK, S. S. Cutaneous absorption. II. The value of petrolatum, anhydrous wool fat and other bases in percutaneous absorption of topically applied cotton seed allergen, *Arch. Dermat. & Syph.*, **49**:427-31, 1944.
167. WERTHEIMER, E. Über irreziproke Permeabilität. I, *Arch. f. d. ges. Physiol.*, **199**:383-401, 1923.
168. ———. Über die Quellung geschichteter Membranen und ihre Beziehung zur Wasserwanderung, *ibid.*, **208**:669-83, 1925.
169. ———. Untersuchungen über die Permeabilität einer isolierten überlebenden Membran, *Protoplasma*, **2**:602-29, 1927.
170. WHITEHOUSE, A. G. R.; HANCOCK, W.; and HALDANE, J. S. The osmotic passage of water and gases through the human skin, *Proc. Roy. Soc. London, s.B.*, **111**:412-29, 1932.
171. WHITEHOUSE, A. G. R., and RAMAGE, H. Permeability of human skin to electrolytes, *Proc. Roy. Soc. London, s.B.*, **113**:42-48, 1933.
172. WILD, R. B. On the official ointments with special reference to the substances used as bases, *Brit. M. J.*, **2**:161, 1911.
173. WILD, R. B., and ROBERTS, I. Absorption of mercurials from ointments applied to the skin, *Brit. M. J.*, **1**:1076-79, 1926.
174. ZHEUTLIN, H. E. C., and FOX, C. L., JR. Use of diffusion rates in evaluating ointment vehicles for antibacterial therapy, *J. Invest. Dermat.*, **11**:161-65, 1948.
175. ZWICK, K. G. A microscopic study of mercury absorption from the skin, *J.A.M.A.*, **83**:1821-24, 1924.

CHAPTER 4

Circulation and Vascular Reactions

I. MORPHOLOGICAL CONSIDERATIONS	61
A. The Cutaneous Vascular System	61
B. Structure of Capillaries	63
II. DIRECT OBSERVATION OF FLOW IN MINUTE VESSELS	64
III. THE COLOR OF THE SKIN AS INFLUENCED BY CIRCULATION	69
IV. HEMODYNAMICS OF CUTANEOUS CIRCULATION	72
A. Capillary Pressure	72
B. Rate of Blood Flow	74
C. Capillary Contractility	76
D. Arteriovenous Anastomoses	77
V. NONNERVOUS VASCULAR REACTIONS	79
A. Constrictor Responses	79
B. Dilator Responses	82
C. Wheal Formation	82
VI. VASOCONSTRICTION OF NERVOUS ORIGIN	85
A. Central Vasoconstrictor Impulses	85
B. Vasoconstriction by Long Reflex	87
C. Vasoconstriction by Local Reflex Mechanism	88
VII. VASODILATATION OF NERVOUS ORIGIN	88
A. The Problem of Sympathetic Vasodilator Fibers to the Skin	88
B. Antidromic Vasodilatation	89
C. Axon-Reflex Vasodilatation	91
VIII. THE TRIPLE RESPONSE OF LEWIS AND THE HISTAMINE THEORY	94
IX. VASCULAR RESPONSES TO THERMAL STIMULI	100
X. VASCULAR RESPONSES TO LOCALLY ACTING CHEMICAL AGENTS	100
A. Metabolites and Hormones	100
1. Acetylcholine	101
2. Nor-epinephrine and Epinephrine	101
3. Pituitrin	102
4. Estrogens	102
5. Male Sex Hormone	103
6. Adrenocortical Hormones	103
B. Pharmacological Agents	104
1. Vasoconstrictors	104
2. Vasodilators	104
XI. REACTIVE HYPEREMIA	105
XII. THE RELATION OF CIRCULATION IN THE SKIN AND IN INTERNAL ORGANS	106

I. MORPHOLOGICAL CONSIDERATIONS

A. THE CUTANEOUS VASCULAR SYSTEM

THE structural basis of cutaneous blood supply in man was established by the early work of Spalteholz (260, 261). As shown in Figure 1, the skin is supplied from the underlying tissue by a large number of arteries. In places where the skin is movable, these arteries are sinuous and able to supply blood even when greatly stretched. They originate from a richly anastomosing, irregular plexus in the deepest part of the corium ("cutaneous arterial network"). From here, single, partly straight, partly arched and branching, arterioles ascend perpendicularly through the corium and anastomose in the upper corium, to form the subpapillary arterial plexus, with oblong meshes more or less parallel to the papillary ridges. The meshes are 0.2–2.0 mm. in diameter, but relatively smaller in regions which are usually exposed to pressure, such as hand and foot.

From the subpapillary plexus, still smaller arteries spring to supply the papillary capillaries (Fig. 2). These "terminal arterioles" do not anastomose. Each of them supplies a small, variable number of papillae. In the plantar region the area supplied by single ascending arterioles is between 0.04 and 0.27 sq. mm.

The capillary loop in the papilla has a narrow arterial limb. The tip of the loop and the venous limb measure 0.02 mm. or more in diameter. The length of a capillary loop is 0.2–0.4 mm. The number of capillary loops per unit area differs greatly in different regions. Wetzel and Zotterman (282) counted 64 per square millimeter on the dorsum of the hand, 47 on the forearm, 16 on the cheek, and 20 on the circumoral region. The small number of capillary loops in the cheek is compensated for by their larger size; but this is not the case for the skin in the perioral region. Nesterow (212) found the following regional differences in number of papillary capillaries per cubic millimeter of skin: hand and forearm, 44; shoulder, 27; chest, 23;

forehead, 25; cheek, 19; lips, 26; thigh, 29; leg and foot, 41.

The venous limbs of the papillary capillaries combine to form venules, which, in turn, form the first subpapillary venous plexus just below the papillae. The veins of this plexus are narrow, their diameters being only a few hundredths of a millimeter. This plexus is connected by short anastomoses with a second venous network lying below the first one in the level of the subpapillary arterial plexus and still consisting of veins with a rather narrow lumen. Deeper in the corium there are two more venous plexuses with large meshes (see Fig. 1). Finally, between dermis and subdermis large veins are seen, some of them having valves.

According to Spalteholz (260, 261), if we disregard the anastomoses between strong arterioles and large veins (true arteriovenous anastomoses, p. 77), the arterial and venous sides of the vascular system communicate only in the papillae. This certainly is not the case. In the fashion of arteriovenous anastomoses on a microscale, terminal arterioles have direct connections with muscular venules, and blood may flow either through these shunts or through the capillary bed (186). Capillary circulation may be present even within the venous system. In deeper layers of the skin Krogh (170) observed small venules branching off from and returning to larger veins, which, being simple endothelial tubes, acted like capillaries. Beyond that, as revealed in the work of Chambers, Zweifach, and their associates (295, 296, 297, 59, 298, 299, 255), the capillary bed of the skin (as well as that of the mesentery, gut, skeletal muscle, and glands) has a number of distinct structural elements which regulate the capillary flow.

Zweifach (299) and more recently Schorr (255) described the architecture of the small vessels (Fig. 3). The terminal arteriole, a vessel with a distinct muscular coat, gives rise to a central preferential or thoroughfare channel, the *metarteriole*. This vessel is sur-



FIG. 1.—A diagram of the skin and its vessels, showing the arrangement of the arterial and venous plexuses at various levels (after Spalteholz). From Lewis (186). (Reproduced by permission of Clinical Science.)

rounded by a single layer of smooth-muscle cells for some length in its course. The channel represents the most direct route from the arterial to the venous circulation. It gives off some offshoots with a few muscle cells around them, the so-called *precapillary sphincters*. They have a strategic location which enables them to control the flow of blood entering the so-called "true" capillaries. The latter arise from the precapillary sphincters, but they themselves are non-contractile endothelial tubes which join with one another to form collecting venules. Blood from the preferential channel and from the capillaries flows into the same venule. Periodic constriction and dilatation of metarterioles and precapillary sphincters constantly affect the capillary blood flow. This arrangement permits complete removal of the true capillaries from the active circulation, without interfering with the flow through the preferential channels.

to the skin as a sign of a relatively low metabolic rate of the skin. However, one should not forget the high proportion of supporting tissue in the skin as compared with that of muscle, which explains this "low-grade me-

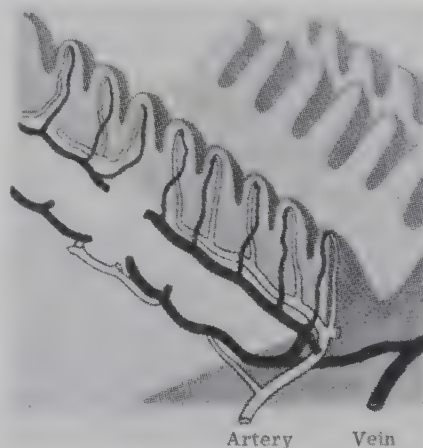


FIG. 2.—Papillary capillaries and subpapillary plexus (after Spalteholz). From Müller (209). (Reproduced by permission of F. Enke.)

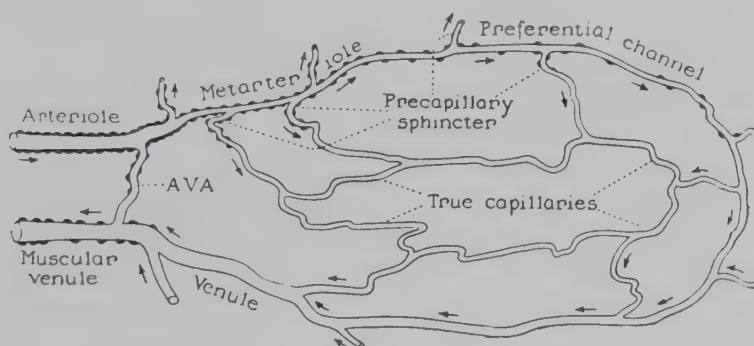


FIG. 3.—A schematic representation of the structural pattern of the capillary bed. The distribution of smooth muscle is indicated in the vessel wall. From Zweifach (299). (Reproduced by permission of the Josiah Macy, Jr.; Foundation.)

The vascular surface available for the exchange of substances between blood and tissue in human skin is relatively small, being somewhere between 1 and 2 sq. cm. per square centimeter of skin surface. Even when the whole capillary surface, including the venous limbs, is assumed to serve exchange of substances, the total "capillary" surface still falls far short of the capillary surface in muscles, and the average distance from tissue elements to blood vessels is much larger in the skin than in the muscles. Krogh (170) regards the low level of blood supply

to the skin as a sign of a relatively low metabolic rate of the skin. However, one should not forget the high proportion of supporting tissue in the skin as compared with that of muscle, which explains this "low-grade me-

B. STRUCTURE OF CAPILLARIES

The knowledge of the structure of simple endothelial capillary tubes and the mechanism regulating their permeability has been greatly promoted by the work of Chambers and Zweifach (60). These authors distinguish three structural components in the

capillary tubes: (1) endothelium per se, consisting of cells in a pavement-like arrangement, connected with one another by an intercellular cement; (2) an inner non-cellular, endocapillary, thin lining film, possibly derived from circulating proteins; and (3) an outer supporting matrix consisting of a delicate sheath of argentophil fibers. There is evidence that the endothelial cells elaborate the intercellular cement and that the cement is continually washed away and reformed. Calcium ions are required for the maintenance of the cement substance in a normal state. Perfusion of the capillaries with fluids free of calcium salts leads to softening of the cement and to a great increase in capillary permeability. Softening of the cement is associated with an increased stickiness, which can be demonstrated by the addition of carbon particles to the perfusion fluid. The particles adhere to the intercellular cement but not to the surface of the cells. Like lack of calcium, lowering of the pH in the perfusion fluid also softens the cement. Distention of a capillary tube involves increased permeability by increasing the pore size in the porous cement material.

The presence of an inner lining is required for the maintenance of physiological permeability. Perfusion of gum acacia solutions, for instance, removes this layer and causes leakage of fluid, with edema formation. The addition of serum to the perfusion fluid restores normal permeability.

The findings of Chambers and Zweifach

(60) made it possible to explain rationally the mode of action of intravenous injections of calcium salts in acute inflammatory skin diseases, a widely used therapeutic procedure. In view of Chambers and Zweifach's work, its effect can be interpreted as restoration of damaged cement substance which serves the "densification" of the capillary vessel wall.

Capillary vessel walls have the physical characteristics of inert nonsecreting membranes, permeable to gases, water, and crystalloids but relatively impermeable to plasma proteins. Landis (177) has calculated that endothelial cells are at least 100 times more permeable to small molecules than are other living cells in the body. Chambers and Zweifach assumed that normal diffusion occurs largely through the intercellular cement substance, which is continually replaced; under pathological conditions, when the cement becomes soft and sticky, the permeability increases to such a degree that proteins can filter through. However, isotope experiments of Cowie, Flexner, and Wilde (73) indicated that water, chloride, and sodium do not escape by way of the intercellular cement alone but that the whole capillary wall is permeable to water and salt. In any case, the structure of capillaries permits them to perform their most important function, viz., outward diffusion of oxygen, water, electrolytes, and other material essential for cellular metabolism and inward diffusion of carbon dioxide and metabolic end-products.

II. DIRECT OBSERVATION OF FLOW IN MINUTE VESSELS

Blood flow through cutaneous capillaries can be observed directly in animals and man by appropriate optical arrangements. In animals the most often studied regions have been the web of the frog's foot and the ear of the rabbit. The latter became particularly suitable for continuous observation through the development of the glass-chamber method by Sandison (251), the Clarks (63), and Ebert *et al.* (10). Frog web and rabbit's ear can be transilluminated through

their entire thickness. In man, so-called "capillary microscopy" is possible only by the use of reflected light. The skin must be covered with oil, in order to create a smooth surface with maximal reflection (209).

In man a truly satisfactory study of capillary circulation is possible only at the base of the nail, because this is the only area in which the papillary capillaries lie horizontally, parallel to the surface, so that the capillary loops can be seen in their entirety with

arterial and venous limb and dome (Fig. 4). Objections have often been made to generalizations drawn from observations on the base of the nail, this area being an "exposed" part of the body surface and being in an "acral" region. It certainly differs greatly in its circulation from that of the trunk and higher parts of the extremities, mainly because of the prominent role of arteriovenous anastomoses in the acral areas (p. 77). Nevertheless, basic physiological facts have

illary microscope *in vivo* is not the vessel proper but the flow of the cellular axis. Changes in caliber of the vessels are assumed when the thickness of this axis changes. Examining the flow at the base of the nail, one sees a rapid and pulsating flow in the arterioles and a very slow flow in the capillaries, with little or no pulsation. In the veins the flow becomes slightly more rapid than that seen in the capillaries. In capillary tubes with diameters larger than that of

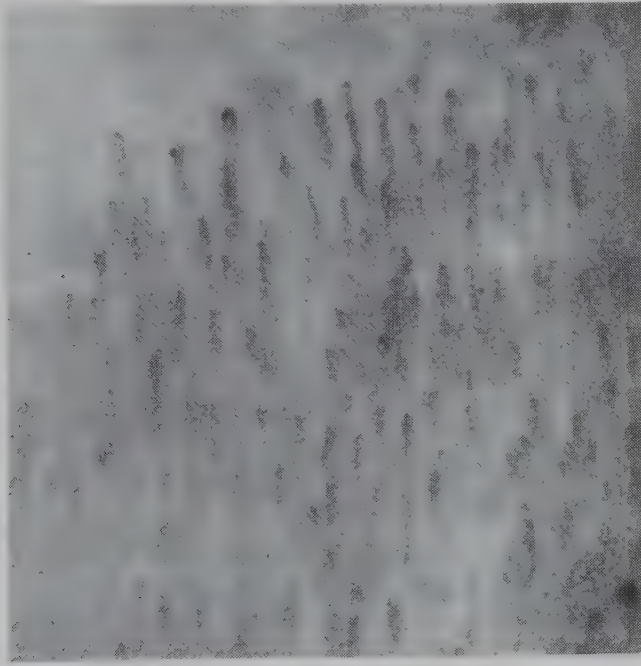


FIG. 4.—Normal capillaries at the base of the fingernail. From Gilje (122). (Reproduced by permission of Dr. Gilje and the American Medical Association.)

been established by observation of the capillary circulation at the base of the nail.

In other parts of the body surface, although the capillary loop of the papilla is not seen in its entirety, capillary microscopy can still be practiced with more or less success, as shown mainly by O. Müller and his group (209). It is true that only the tip of the dome of the loop is visible in those capillaries which stand with their long axes at right angles to the surface. However, many of them are lying in an oblique direction, so that parts of the loop may be seen by direct observation.

As pointed out by Krogh (170) and others, what is actually observed in the cap-

two red corpuscles, the erythrocytes flow in an axial stream. Occasionally leukocytes can be seen outside the axial red stream, flowing with rolling movements. In veins a peripheral plasmatic and central erythrocytic zone can be clearly distinguished; scattered leukocytes are found here with greater regularity, traveling outside the axial stream more slowly than red cells. In all larger vessels it can be seen that the axial stream of red corpuscles is laminated concentrically; the central lamina passes along most rapidly, and each additional one-cell layer passes more slowly than the one inside it.

In capillaries which are so narrow that red cells contact the wall, there is no separa-

tion of the stream into zones. The corpuscles often pass in single file and are squeezed out of shape as they progress. Krogh (170) studied in detail the deformations of red corpuscles under such circumstances, as well as at vascular arborizations, and found that they have a high degree of plasticity and elasticity. Complete blockage of capillaries by red cells does occur but lasts only a short while.

In single capillaries the flow becomes retarded and accelerated. The flow may stop entirely for from one to several seconds, either simultaneously in all capillaries of the microscopic field or only in small groups or even in single capillaries (209). Wherever the flow is greatly slowed down, the corpuscles, having no plasma between their surfaces, become agglutinated by mutual attraction and adhesiveness. This is counteracted by a faster flow, as soon as it sets in. The packing of corpuscles at a temporary stasis of flow is more frequently seen in the frog than in man.

Sometimes a capillary appears to have a "granular" content and then suddenly "disappears," indicating that it has emptied its contents; but soon it is filled up again (209). O. Müller (209) found that this phenomenon occurs rhythmically in capillaries of the nail base in man.

In inflamed tissues the capillaries are maximally dilated, the flow is slowed down, and diapedesis of erythrocytes and migration of leukocytes through the vessel wall can be observed. The red corpuscles slip through the wall smoothly as if there were a hole; sometimes they first bulge the capillary wall and then are suddenly seen outside the vessel. The integrity of the wall, however, is restored rapidly: after a few seconds red corpuscles pass intravascularly past the point where diapedesis was observed before. Leukocytes first become adherent to the wall, then change their shape, and slowly pass through the endothelium by means of pseudopods. This is active penetration, as contrasted with the passive diapedesis of red corpuscles. As after extravasation of red corpuscles, the wall is readily restored after penetration of leukocytes through the wall.

Intravascular formation of red corpuscle "sludges" was studied in great detail by Knisely and co-workers (163) as an anomaly occurring in a great number of pathologic states in man, including practically all severe systemic diseases. Most observations were made on the obliquely illuminated bulbar conjunctiva, but there is evidence that the same phenomenon also occurs in cutaneous capillaries and in capillaries of internal organs.

Essentially, the sludge formation represents an intravascular agglutination of red corpuscles, based probably on the formation of an abnormal protein coating around them, whereby their surfaces become sticky and cell masses are formed. The agglutinated masses may move along with the plasma, but they also may settle and intermittently shut off small vessels. The coated cell masses are easily ingested and destroyed by phagocytes. In any case, resistance of sludged blood to its own flow may considerably reduce the rate of flow. Among diseases which are of dermatologic interest, extensive burns, different forms of shock, Buerger's disease, granuloma inguinale, malignant hypertension, and even half of all normal pregnancy cases show sludged blood in the capillary vessels.

Stickiness of blood cells can also be provoked by experimental injury. The Clarks (65) have shown for the capillaries in the rabbit's ear that in intact vessels red corpuscles and white cells do not stick either to each other or to the capillary wall. Under the influence of graded injury, however, first single leukocytes and later whole layers of them stick to the wall. In higher degrees of injury, when the capillaries become dilated, bulged, sacculated, and leaky, the red corpuscles also become sticky, and the vessels are packed with masses of agglutinated erythrocytes. Recently, Ebert and Barkley (93) studied these phenomena in detail in acute and chronic inflammatory processes.

A most important general feature of capillary circulation is the permanent variation in the number of open capillaries in a given field of observation. Some widely opened

capillaries, in which the flow was rapid a moment earlier, disappear from the field, and at the same time capillaries hitherto invisible suddenly spring into view. Krogh (170) was the first to emphasize the functional role of these changes in the regulation of the oxygen supply to the tissues. In tissues and organs at rest, the majority of the capillary vessels are closed, whereas during activity all or most capillaries open up. Starving cells will obtain their nourishment when the nearest capillaries open up. The variation in number of open capillaries has been observed in animal and human skin, as well as in most internal organs studied. While Krogh (170) interpreted these changes as due to active capillary contraction, Chambers and Zweifach (59, 299) emphasize that capillaries behave like passive tubes. The circulation of blood in them depends on periodic contractions of the pre-capillary sphincters. Clark and Clark (66) found less conspicuous spontaneous contractions of minute vessels in the rabbit's ear than Krogh (170) and his group did.

The view that there is a great variability of circulation in human cutaneous capillaries, due to alternate opening and closing of single branches, was opposed by Lewis (186). He was of the opinion that in human skin a decrease or increase in rate of flow is obtained by change of flow "through the capillaries as a whole, rather than by opening of additional or closing down of redundant capillary channels." He thought that in the human skin the minute vessels always act together as a physiological unit and that, when "opening" and "closing" of individual capillaries is spoken of, too much prominence is given to small and particular elements picked out of the complex meshwork of minute vessels. Lewis even thought that possibly human skin capillaries have a unique position because, in addition to the nutrition of tissues, cutaneous capillaries also have the important function of dispersing heat from the body surface. From this point of view, said Lewis, it might be more advantageous to have many capillaries open all the time. As contrasted with data of

Krogh's school, Lewis found only about 12 per cent of the cutaneous capillaries invisible at a time.

Notwithstanding Lewis' arguments, the conspicuous and continual opening and closing of the single capillary loops in the papillary bodies of human skin has been reported on the basis of direct observation so frequently (57, 38, 209) that there is no reason to believe that circulation in the papillary capillaries in man is basically different from that in other mammals. Bordley *et al.* (38) observed marked intermittency of flow in the capillaries of the skin over the tibia in man. They even saw two capillaries arising from the same arteriole alternate with each other in permitting the passage of red corpuscles. Findings on changes in the number of open capillaries following vasodilator

TABLE 1

	Before Bath	After Bath
Upper arm.....	8-10	20-40
Thigh.....	20-30	60-80

stimuli in human skin also militates against the view of Lewis. For instance, O. Müller (209) quoted data on the number of open capillaries before and after a warm carbon dioxide-water bath in the natural spa spring water of "Nauheim Sprudel" (Table 1).

In acute inflammatory processes, all visible minute vessels, papillary capillaries, and subpapillary plexuses are wide open, the flow is rapid, and the background of the microscopic field has a diffusely red tinge (209). In cyanotic skin, for instance in acroasphyxia or in perniosis, the subpapillary venous plexus is enormously dilated. The dilatation extends up to the venous limb of the papillary-capillary loop. The arterial limb and the arteriolar plexus are narrowed. Complete cessation of flow alternates with slow, sluggish, or even pendulum-like movements (98).

The capillary-microscopic picture of telangiectases (and even of uninvolved skin in cases of essential telangiectasia) shows tor-



FIG. 5.—Nail-fold capillaries in lupus erythematosus. From Gilje (122). (Reproduced by permission of Dr. Gilje and the American Medical Association.)



FIG. 6.—Nail-fold capillaries in polycythemia vera. (Courtesy of Dr. Gilje.)

tuosities and dilatations of small vessels (200, 257). Lichen pilaris is often associated with cyanosis, particularly in the cold. Apparently, this cyanosis is caused by pressure exerted by the keratotic follicular plug on the perifollicular vessels. It has been claimed (209) that the capillary-microscopic picture of such areas is characterized by a dilatation of the dome of the papillary-capillary loops.

Many attempts were made to utilize capillary microscopy in the diagnosis of diseases in general (209) and of diseases of the skin in particular. The latest and probably most successful work in this field has been that of Gilje (120, 121, 122). He found rather characteristic changes of the nail-fold capillaries in lupus erythematoses. As contrasted with the picture in normals (Fig. 4, p. 65), the loops are uneven in height and shape, both arterial and venous limbs are greatly dilated (Fig. 5) and the stream is slow and granular. Such changes in the nail-fold capillaries have been present not only in the disseminate systemic disease but also in the discoid form of lupus erythematoses. Thus, even chronic

localized lupus erythematoses may well be a general vascularized disease. The capillary-microscopic finding fits the observations of Huff, Taylor, and Keys (152), who found slow reflex vasodilatation, increased crest times, and obliteration of the diastolic notches with the photoelectric plethysmograph in the chronic form of this disease; and they concluded that the chronic form, too, may be accompanied by general vascular changes.

Another example of characteristic pathological findings is the picture in psoriatic plaques as described by Gilje (120, 121, 122). Here the tips of the capillary loops in each papilla are curled into balls. In lichen planus papules an irregular central field is surrounded by regularly arranged, hairpin-shaped, slender, slanting capillary loops. In polycythemia vera a peculiar elongation, loop formation, and curving of the nail-fold capillaries was observed (Fig. 6). Gilje has utilized the capillary-microscopic technic also for study of the surface relief of the skin (120, 121, 122).

III. THE COLOR OF THE SKIN AS INFLUENCED BY CIRCULATION

The color of the skin surface¹ is determined by the color of the surface layers of the epidermis, by the amount and distribution of pigments, and—particularly in the white race—by the filling of the capillary vessels. The contribution of the circulating blood to the color of the surface is easily demonstrated when a circumscribed spot is made anemic by glass pressure (“diascopy”) or by introduction of a drop of dilute epinephrine solution. If this is done, a creamy-white color results which is distinctly different from the color of normal skin.

The amount of filling of the papillary capillaries contributes little to the color, partly because of their relatively small extent, partly because only the dome of the loop faces the surface. Decisive for the color is the state of the subpapillary-capillary plexus. This is a rich, dense conglomerate of

capillary vessels in which blood flows predominantly parallel to the surface.

The tint caused by increased filling of the subpapillary capillaries depends on the oxygen saturation of the blood. The tint is vivid red (“flushed skin”) when the blood is well oxygenated, but cyanotic if oxygen is lacking. Whether the vivid red color is different when, in addition to the capillaries, superficial arterioles also dilate has not been established, but there is some evidence (p. 80) that the red color deepens with additional dilatation of arterioles.

It is an important and often emphasized fact that no conclusions can be drawn from the skin color about either the rate of blood flow or the temperature of the skin. Redness or paleness of the human skin depends upon the quantity of blood *present* in the subpapillary plexus, i.e., upon whether the vessels are filled or empty, while the temperature of the skin depends chiefly on the rate

1. See also chap. 22.

of flow through the whole skin. The skin can be warm and pale. This is the case when arteries, arterioles, and venules are dilated but the subpapillary capillaries are not. In such situations the blood flow through the skin is increased many fold, and the surface temperature is considerably elevated, but there is still no visible hyperemia. Blood



FIG. 7.—Mottling (“cutis marmorata”)

may flow through the skin very fast, with only the preferential channels of the capillaries (299) being open or even faster through the shunts between metarterioles and venules. If more or all of the precapillary sphincters open because of high pressure from above or for some other reason, blood will rush through the whole subpapillary-capillary bed, and the skin will become red. The rate of blood flow and the skin temperature, however, are not substantially influenced by the capillaries being open or closed.

On cooling, arterioles and metarterioles are contracted, but many capillaries and venules are open. In this case the skin becomes cyanotic because the flow is slow and the oxygen of the blood is used up to an unusual extent. On exposure to more intense cold, however, when there is complete stoppage of flow with maximum opening of the capillaries, a flushed color may result because the metabolism of the skin tissue is depressed so much by the cold that there is no appreciable consumption of oxygen (186). Thus the same flushed color may be present at rapid flow (in flushed and warm skin) and in complete stasis (in flushed and cold skin).

When blood is pressed out from a cyanotic area and anemia has resulted and when, afterward, the pressure is released, the cyanotic color recovers slowly by spreading from the periphery to the center. This is in contrast to the momentary and complete re-reddening of spots which, before pressure was exerted, were in a state of active hyperemia with increased blood flow and open capillaries (186).

Mottling, consisting of irregular red and white spots, is sometimes seen in different skin areas, most frequently on the palms. While earlier authors believed that the sites of anemic and hyperemic spots in this mottling change continuously, corresponding to an alternating opening and closing of capillary circuits, Lewis (186) emphatically claimed that red and white spots have a very constant position. He observed mottled areas for many months, charting the pattern of mottling, and found that white spots may become pink or red but that later the pallor returns and has the same contours as it had before.

Some persons display a special kind of mottling on trunk and/or extremities, consisting of rhomboidal, square, or irregularly shaped pale spots, each several centimeters in diameter, encircled by a darker reticulum, the lines of which are 1–3 mm. in width (Fig. 7). This mottling was called “cutis marmorata” because it was reminiscent of the surface pattern of marble. It is more marked in cool environment: in the cold, the

meshwork becomes either slightly pink or, more often, cyanotic. The reticulum corresponds to areas in which the capillaries are relatively more dilated than in the pale spots. Again, in the studies of Lewis (186) this pattern showed considerable stability.

The reticulum seems to be more vulnerable to inflammatory stimuli than do the paler spots in between, possibly because of the more open state of the vessels in the reticulum. The reticular type of inflammation was called "livedo racemosa" or, better, "inflammatio cutis racemosa" (183). Furthermore, the reticulum has a greater tendency to become pigmented, with or without

In contrast to those forms of mottling which are obviously based on a developmental anomaly of the cutaneous vascular system and are not connected with any particular disease, there is a form, usually called "livedo reticularis," which develops in adult life, chiefly on legs and feet, as a consequence of arteriolar disease. Barker *et al.* (19) discussed this condition in detail, and a great number of cases of this kind has been reported since their publication (236, 216, 256). "Development" of cutis marmorata in caisson disease was reported by Aldao (12). Such "development" either occurs in persons who have latent cutis marmorata when their venous pressure is increased or could



FIG. 8.—Bier's white spots. A pressure of 30 mm. Hg having been thrown upon the veins of the forearm for $\frac{1}{2}$ minute, the circulation to forearm and hand was then stopped by forcing the armlet pressure far above systolic blood pressure. The photograph shows the condition of the arm 20 minutes later. Room temperature 19° C. The skin of the forearm and hand below the armlet was, for the most part, deep purple in color and presented innumerable white spots, irregular in size and form. The small dark spots are freckles. From Lewis (186). (Reproduced by permission of Clinical Science.)

preceding inflammation. The pattern of reticular pigmentation corresponds exactly with the pattern of deeper vascular coloration which becomes masked by the pigmentation. It has not been investigated whether or not this pigmentation is caused by the formation of new melanin granules. The most common cause of reticular pigmentation is heat. This and the fact that pigmentation occurs in areas which presumably are better oxygenated make me believe that this type of pigmentation is brought about by oxidative pigment darkening (Meirowski phenomenon, p. 522) rather than by true pigmentation. However, the increased temperature of the inflamed reticulum may possibly promote true melanin production also.

be a consequence of arterial embolization by gas.

If the circulation to a limb is arrested, in the diffusely cyanosed skin there appear irregularly shaped, pale spots (so-called "Bier spots"), bright-red spots, and "cinnabar-red" spots (Fig. 8). Lewis (186) dealt with this phenomenon and found that the pale spots are due to some vasoconstrictor substance which is formed in tissues deprived of oxygen. The vascular contraction is independent of nervous influences and appears even if the blood volume is greatly reduced (245). The capillary contraction seems to serve improved blood supply to vitally important tissues in emergency situations (245). Blood circulation in the red spot originates from noncompressed vessels of

the bones, which anastomose with cutaneous vessels (226). The capillary-microscopic picture of these spots is characterized by fairly

good flow through moderately opened arterioles and a widely open subpapillary venous plexus (217).

IV. HEMODYNAMICS OF CUTANEOUS CIRCULATION

A. CAPILLARY PRESSURE

Large arteries represent elastic conduits of blood circulation. Small arteries, arterioles, and the metarterioles and precapillary sphincters of Chambers and Zweifach (p. 61) have the additional function of regulating the amount of blood flowing through the capillary area they supply. This function is achieved by contraction and relaxation of the muscular elements of these small arterial vessels.

The fall of pressure from the aorta to the arterioles is only 20 mm. Hg or less. During the passage of the blood through arterioles the drop in pressure amounts to 50–60 mm. Hg. The further drop beyond the arterioles is due mainly to the sudden increase of the surface area of the total vascular bed. When many capillaries are open, the cross-sectional area of the capillary bed is up to 800 times greater than in the aorta. This enlargement of the vascular bed causes considerable slowing of the rate of blood flow. If all capillaries open up at once, as is the case if the vasomotor center is destroyed, the capillary area increases, and the rate of flow diminishes to such a degree that return of the blood to the heart becomes impossible and shock results (106). In the collecting venules and veins the pressure continues to drop.

Even if the total cross-sectional area of the open capillaries were not much greater than that of the aorta, they still would cause a marked fall in blood pressure because they are the site of considerable frictional resistance.

Zweifach (299) pointed out that the metarterioles, with their single layer of smooth-muscle cells, constitute an intermediate zone in the vascular system, being, on the one hand, under the influence of systemic factors such as blood pressure and arteriolar resistance, regulated mainly by

the sympathetic nervous system, but, on the other hand, also under the influence of local factors which regulate capillary circulation. He also emphasized that precapillaries are given off mostly at an acute angle and frequently branch in a backward direction; that most precapillary branches are coiled and tortuous; and that this situation promotes the tendency to break the blood flow before it enters the capillary bed.

The blood pressure in the capillaries is one of the physical factors determining their permeability. Theoretically, the pressure would be expected to filter fluid from the blood through the capillary wall into the extravascular spaces. However, as was assumed by Starling as early as 1896 (263), the colloid osmotic pressure of the blood counteracts the capillary pressure. When capillary pressure exceeds the colloid osmotic pressure of the blood, fluid is filtered from the blood into the tissue spaces. When capillary pressure falls below the colloid osmotic pressure of the blood, fluid will be absorbed from the tissue spaces into the circulating blood.

Capillary blood pressure can be measured directly and indirectly. The direct method consists of inserting micropipettes into individual capillaries under the control of a micromanipulator and connecting the other end of the pipette with a water manometer. This direct microcannulation method was first applied by Carrier and Rehberg (58), greatly perfected by Landis (174, 175, 176), and is the only truly reliable method (95). Unfortunately, in man only the nail-bed capillaries are accessible to cannulation, and these vessels are probably not representative because of their continuous exposure to external factors (99). Also, only a few capillaries can be pierced in the course of an hour or so, and averages are unjustified unless large numbers of determinations have been

made within a short time (177). Still, Landis succeeded in establishing the range of pressure in human skin capillaries as being between 12 and 45 mm. Hg.

The indirect methods for measuring capillary pressure are based on the principle of applying graded pressure to the tissue and establishing a state of balance between external pressure and intravascular pressure. The criteria for the establishment of this equilibrium have been arbitrary. For the human skin, first perceptible blanching, definite blanching, or stoppage of flow in capillary vessels viewed under the microscope were used. The results obtained with the indirect method have been extremely variable (1.5–71.0 mm. Hg), and the usefulness of such measurements has been questioned.

Landis (176) found in four different species, including man, that the fall in blood pressure, as the blood is carried to the periphery, does not cease at the junction of the arterioles and capillaries but continues through the capillary network, being measurably greater in the arterial than in the venous limb. In normal human skin Landis' average pressure values were 43.5 cm. water for arteriolar capillaries and 16.5 cm. water for venous capillaries. In hyperemic skin the arteriolar capillary values were between 71 and 93 cm. water, and venous capillary values were between 54.5 and 66.5 cm. water.

The average capillary pressure is approximately equivalent to the colloid osmotic pressure of plasma proteins. Owing to the pressure gradient within the capillaries, it seems that under average conditions filtration of fluid from the capillaries into tissue spaces is favored in the arteriolar limb, while absorption of fluid from the tissue into the capillary vessel is favored in the venous limb. Obviously, the very high capillary pressure in hyperemic skin greatly promotes the outward filtration of water, with resulting hydration and possibly visible edema of the tissues.

The variability of capillary pressure, in the same capillary from moment to moment

and in adjacent capillaries at the same time, is very great. The pressure depends, among other factors, on the arteriolar tone (arteriolar constriction increases the peripheral resistance and thus lowers capillary pressure), on freedom of venous outflow (a rise of venous pressure elevates capillary pressure), on posture (capillary pressure is high in dependent parts of the body because the venous pressure is high in the vertically placed veins), on temperature (local heat causes hyperemia), and on the state of innervation. Zweifach and Chambers (59, 299) put the main emphasis on the behavior of metarterioles and precapillary sphincters. When they are dilated, the head of pressure will be higher, and outward filtration will predominate. When they are constricted, pressure will fall, and inward filtration will prevail.

Posture, of course, is an important factor. Capillary pressure in the skin of the fingers in man may vary between 40 and 4 cm. water, according to whether the hand is hanging or raised. But however high up the hand is placed, the pressure never falls to zero or becomes negative, because when the hand is held high and the venous pressure becomes negative, the veins collapse and restrict the flow (106). In the feet the capillary pressure does not attain high values because of the presence of valves in even the smallest veins and because of constant small muscular movements which, by forcing the blood along these veins, enable them to act as a kind of pump. The wide variations in capillary pressure bring about gross disproportions in the relation of colloid osmotic pressure and blood pressure, alternately favoring massive filtration from, and massive reabsorption into, the capillaries (177).

Landis' results perfectly fitted Starling's concept. However, Rous *et al.* (247) found that a dye such as trypan blue, when injected into the vascular bed, concentrated in the tissues near the venous end of the capillary. Therefore, they thought that outward filtration was greatest at this point. To reconcile this finding with Landis' results, Chambers and Zweifach (60) sug-

gested that the pressure in the thoroughfare channels was higher than that in the true capillaries and that in Rous's experiments outward filtration occurred through the thoroughfare channels. In the true capillaries filtration might be outward or inward according to the pressure in the arteriolar end and inward at the venous end.

B. RATE OF BLOOD FLOW

There are three methods for study of the blood flow in the skin (51).

1. The first method is direct observation of the flow under the capillary microscope and determination of the time taken by any given single red cell to travel a measured distance. In man such study is easily made only at the edge of the nail.

2. Determination of blood flow with the pressure plethysmograph is the second method (49). It is based on the principle that, if the venous return is occluded immediately before the measurement, changes in volume represent the amount of blood which has entered the part inclosed in the plethysmograph. The duration of observation must be necessarily short because interference with the venous flow—the occlusion of veins—slows the blood stream, and fallacious results are obtained unless only instantaneous values are recorded. Furthermore, to obtain information on cutaneous flow, this method can be applied only to fingers, toes, and ears, where volume changes are due almost exclusively to changes in circulation of the skin.

3. The third method uses oscillometric technics, by which cutaneous arterial flow can be measured. If the venous pressure and heart rate are constant and the capillary tone (p. 76) is not altered, a change in volume or volume pulsation of the cutaneous vessels is a measure of arterial flow. Hertzman's photoelectric plethysmograph (144, 145) takes advantage of the fact that absorption of light by a transilluminated tissue varies with its blood content. In his ingenious arrangement, skin can be transilluminated, and light which has passed through the skin reaches an attached photocell. Light scat-

tered from the surface does not enter the photocell. This method has replaced all other plethysmographic procedures. It has the great advantage that it makes possible the measurement of blood flow anywhere on the skin surface.

There is a moderate decrease in the velocity of flow when blood passes from the arteries to the arterioles. A sudden and steep drop in the rate of flow occurs when blood enters the capillaries, mainly because of the sudden increase in the cross-sectional area. In the collecting venules and veins the blood flows faster again, but still much slower than in the arterioles.

The main result of measurements of blood flow through the skin has been its extreme variability in an amazingly wide range. In addition, it was found that cutaneous vessels of different regions respond differently to identical stimuli. The variability of the flow is obvious from direct observation. Not only is the flow faster in arterioles than in veins and slowest in the capillaries, but there are continuous changes within the capillaries, with a constant quickening and slowing of the flow. The rapidity of the flow depends primarily on the state of contraction of arterioles, metarterioles, and precapillary sphincters. The arterial pulse wave reaches the arterioles but is rarely seen beyond them. In the presentation of Zweifach (299) the terminal arterioles are the major factors in converting the pulsating flow to a continuous stream. Precapillary sphincters further dampen the pulsation, so that even under the conditions of arterial hyperemia no pulsation is seen in the capillaries. However, in the preferential channels (p. 61), there may be pulsation when there is arteriolar dilatation.

Plethysmographic measurements in fingers reveal that the range of variations in blood flow is more than two hundred fold. The minimum value with maintained vasoconstriction is about 0.5 cc. per minute per 100 cc. of tissue, while in maximal vasodilatation the corresponding value is 100 cc. per minute per 100 cc. of tissue (285, 49). The variability is based on three main

mechanisms: (1) isolated contractions of arterioles and metarterioles due to "intrinsic" contractility or to periodic accumulation of some chemical agent; (2) sudden decrease of flow in acral parts, such as fingers, toes, and nostrils, under the influence of rhythmic central sympathetic discharges, occurring every 30–40 seconds (53); the average flow is established not by a steady vascular tone but by a rhythmic swinging between constriction and dilatation; and (3) the flow varies one hundred fold or more by the opening and closing of the large arteriovenous anastomoses (p. 77).

The periodic vasomotor activity in the terminal vascular bed has been called "vasomotion" by Chambers and Zweifach (299). They find that it is based on periodic contraction and relaxation of metarterioles and capillary sphincters. The periods last from 30 seconds to 3 minutes. In nonmuscular collecting venules, both the narrowing and the dilating periods last longer. There may be increased or depressed vasomotion, with either the constriction or the dilatation being more prominent. When constriction prevails, there is low hydrodynamic pressure in the capillaries, and inward filtration predominates, so that tissues dehydrate; while with prevailing dilatation the situation is reversed, and hydration of tissues takes place. Vasomotion is probably under both local and nervous influences. Denervation stops vasomotion, but about 48 hours later it returns. After sympathectomy the denervated vessels become highly sensitive to both constrictor and dilator stimuli. Contraction can be produced with epinephrine in dilutions as high as 1:200,000,000. The nervous influence seems to extend only to vessels with muscular elements.

The abundance of arterial supply to acral parts, with a corresponding great variability of flow, shows their role in regulating dissipation of heat from the surface and in providing rapid vasomotor adjustments for distribution of blood to other organs according to need. Hertzman (145) finds the following descending order of arterial supply in the areas studied: finger pad, ear lobe, toe

pad, palm of hand, skin of forehead and face, dorsum of finger, dorsum of hand and foot, forearm, knee, tibia.

Regional differences in vascular responses to similar stimuli can be best illustrated by comparing the reactions of the skin of hands and forearms, respectively (111). Painful stimuli, deep inspiration, mental activity, emotional stimuli, and tobacco inhaling cause active arteriolar constriction in the hands (more in fingers than in other parts of the hand), while at the same time blood flow to the forearm is not influenced at all or only insignificantly. Stimuli increasing systemic blood pressure (carbon dioxide inhalation, hyperventilation, epinephrine) elicit vasoconstriction, with diminished flow to the hand but increased flow to the forearm. In reactive hyperemia (p. 105) the hands repay only a small percentage of the circulatory debt because of interference of sympathetic vasoconstrictor impulses, while hyperemia in the forearm equals, or more than compensates for, the debt (111). In response to heating, arterioles of both hands and forearms dilate, but this reaction develops much faster in the hands and at lower temperatures than in the forearms (129). Although the forearm contains only 13.4 per cent skin as compared with 30 per cent in the hand and 50 per cent in the fingers, still the skin of the forearm contributes a sizable portion of the total flow at average atmospheric temperatures (171). It appears that the main reason for the different behavior of hand and forearm is the presence of arteriovenous anastomoses in the hands (130).

A conspicuous regional difference in vascular responses is that connected with emotional blushing. This patchy vasodilatation is confined to the head, neck, and thorax, and I saw it occurring also on the upper back in a few cases. Lewis (186) found it to be restricted to exposed areas and assumed that arterioles in chronically exposed areas behave differently. In any case, whatever the reason for the restricted localization and whatever the mechanism (sympathetic paralysis or antidromic vasodilatation, p. 89),

it is associated with vasoconstriction in the digits. The same paradoxical reaction is observed also after administration of amyl nitrite. Burton (51) points out that, in view of such paradoxical reactions, it is not necessary to assume either reciprocal nervous effects or differential chemical actions on different vessels. It seems that there are several nerve impulses set up at the same time, and, in addition, the response of a particular vessel depends upon the relative degree of nervous and of chemical control of the vessel. In the finger, vasoconstrictor effects predominate, while in the face vasodilatory reactions arise with particular ease.

C. CAPILLARY CONTRACTILITY

For a long time it was thought that all capillary vessels have the intrinsic ability to contract and to expand, independent of hemodynamic forces, such as the arteriolar pressure, and independent of nervous influences. The contractility, first postulated by Ebbecke (92) in 1917, was attributed to the presence of differentiated contractile cells, the so-called "Rouget cells," which contain fine contractile fibrils and form a wide-meshed network around the endothelial tube (276, 277, 170). Krogh suggested that although the caliber of capillaries is, to some degree, affected by the blood pressure on the capillary wall, normally this effect is very small, partly because of the relatively large integral transverse section of the capillary bed and partly because the capillaries withstand pressure remarkably well. A venous pressure of 30 cm. water, for instance, is withstood for several minutes by the capillaries before they dilate slightly.

Whereas Krogh attributed all capillary contraction to the Rouget cells and denied that the endothelial cells themselves are contractile, Ebbecke (92) and O. Müller (209) claimed that these cells are able to change their volume or even to move actively with "ameboid" movements. Beecher (30), working in Krogh's laboratory, suggested that, in addition to constriction effected by the Rouget cells, capillaries may contract by swelling of the nuclei of endo-

thelial cells. Actually, reversible contractions of these cells were observed by Chambers and Zweifach (60) under the influence of hypertonic solutions. The nature and role of Rouget cells, however, are doubtful. The Clarks (62) and Sanders, Ebert, and Florey (250) found that Rouget cells did not participate in the contraction of either mammalian or amphibian capillaries.

The more recent work of Chambers and Zweifach (59, 298, 299) led to the well-evidenced conclusion that the true capillaries, simple endothelial tubes, never undergo active vasoconstriction. According to these authors, what has been described as Rouget cells seem to be the muscular cells of the preferential channels (p. 61). But beyond the precapillary sphincters there is no muscle element (see Fig. 3, p. 63), and true capillaries show no changes in caliber attributable to pericapillary muscle cells either in amphibians or in mammals. They find that in the resting state there is intermittent opening and closing of precapillary sphincters, with blood flowing now through one, now through another, capillary. When the capillary sphincters are constricted, the capillaries are ischemic, and blood flows exclusively in preferential channels. In hyperemia there is accentuation of the dilatory phase of precapillary sphincter activity (instead of periodic changes), and this results in an over-all blood flow through the entire capillary bed (Fig. 9).

Endothelial cells do have a cell tone which is essentially an elastic property, permitting resistance to distortion. It is because of this tone that capillaries become somewhat narrow when blood does not circulate in them. The normal tone returns when blood enters them again. Vasodilatation caused by certain noxious agents, such as urethane, is the direct result of loss of tone in the endothelial tube. When the caliber decreases, the endothelium can be seen to thicken: the nucleus bulges into the lumen. However, such caliber changes in the capillaries are minor, not very evident, and always slow. Normally, the diameter of a capillary is 2-3 μ greater than that of red cor-

puscles, which pass in single file. Narrowing of the capillary would cause distortion of the corpuscles; but this is a very rare event. In vessels with muscular elements the changes are rapid and sudden, and one can easily see how their contraction changes the direction of blood flow. Therefore, it is doubtful whether one should talk of "contractility" of capillary tubes, even if there is an atonic

changes in capillary flow secondarily. Metarterioles and precapillary sphincters are particularly sensitive to nonnervous stimulation, for instance, by epinephrine, nor-epinephrine, and substances arising locally in tissue metabolism (pH, electrolyte shifts, other metabolic products), while sympathetic nerve impulses act primarily on arterioles. It is highly improbable that non-

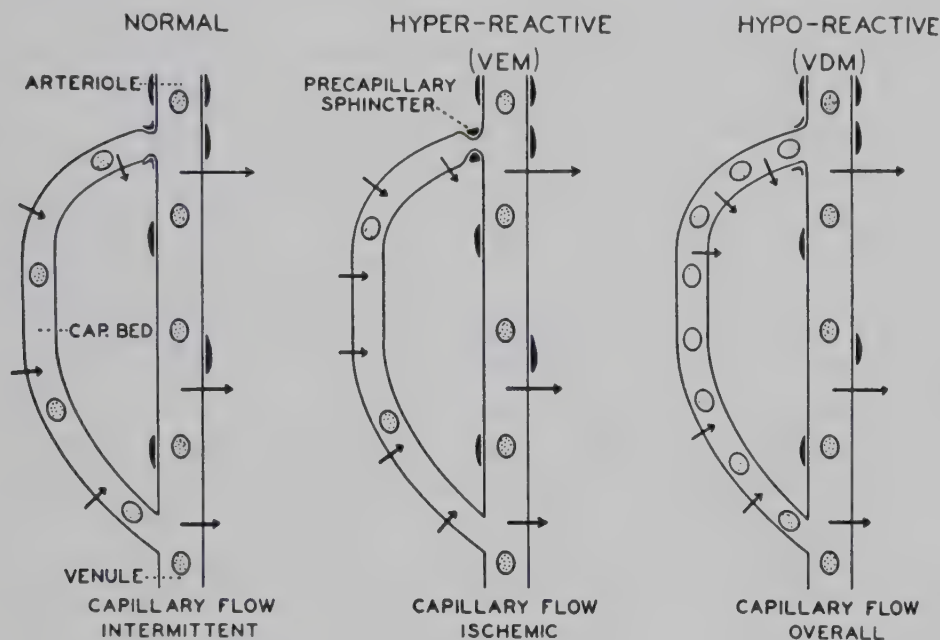


FIG. 9.—Diagram of fluid exchange between blood and tissues. The number of stippled cells in the side branch indicates the number of open capillary channels. The arrows indicate the preponderance of fluid movement, the length of the arrow giving an approximation of the magnitude of fluid movement in comparison to other capillary vessels. From Zweifach (299). (Reproduced by permission of the Josiah Macy, Jr., Foundation.)

state of capillaries from which they slowly return to the state of normal tonicity. The decrease of capillary tone has great physiological significance, not for the distribution of blood but for influencing the permeability characteristics of the capillaries.

This view has been brilliantly substantiated by an enormous number of direct observations in combination with micromanipulatory operations by Chambers and Zweifach. They postulate that capillary circulation is under the control of muscular parts of the system, terminal arterioles, metarterioles, and precapillary sphincters. These are the elements which receive nervous and nonnervous stimuli and cause the

muscular parts of the capillary tubes are innervated at all.

D. ARTERIOVENOUS ANASTOMOSES

Clark (61) reviewed in detail our knowledge and its historical development in this field. Arteriovenous anastomoses constitute direct connections between arteries and veins, with calibers considerably greater than those of capillary vessels. When they are open and the artery or arteriole is contracted distally from them, the capillary circulation is completely by-passed. Most of them consist of three portions: an arterial (or arteriolar) portion; a thick-walled intermediate portion, which, in addition to the

usual circularly arranged muscle fibers of arterioles, also contains an inner longitudinal and an outer oblique muscle layer with an "epitheloid" appearance; and, finally, a venous portion. On direct observation one can see that the intermediate portion has the most active contractility. The arterial portion is often twisted or coiled, the venous portion usually is funnel-shaped. The shunts may occur as single or double connections or as "bouquets" of multiple anastomoses. They are richly innervated by sympathetic (and sensory?) fibers. The whole twisted structure usually forms a sort of ball or "glomus." In the fingers and toes of man their internal diameter at the narrowest point is usually 20–40 μ and rarely exceeds 50 μ .

In the skin of mammals these structures have been consistently found in the terminal phalanges of forefeet and hind feet, in the ears, and in the nose or snout, in some animals also in the tips of the tail, a conspicuously acral distribution, corresponding with those parts which are most likely to freeze in extreme cold. In human skin they have been found consistently only in the nail folds, in the pads of fingers and toes, and in palmar skin. Their occurrence in other acral regions of man has been described but requires further confirmation. In fingers their distribution is typically acral: Grant and Bland (127) counted 501 per square centimeter on the nail fold of the index finger but less than 100 on the proximal phalanx and on the palm.

Direct evidence in animals and indirect evidence in man (127) are conclusive that the arteriovenous anastomoses of the fingers are active in contraction and dilatation and that they play an important role in the temperature changes of the skin of the fingers. When blood flows mainly through the shunts instead of through the superficial capillary bed, less warm blood is brought close to the surface, and there is less cooling of the blood. At the same time, the arteriovenous anastomoses can protect peripheral areas from overcooling, because, if they are

open, the rate of blood flow is greatly increased. It was calculated that at least 256 times as much blood will pass through an anastomosis 40 μ in diameter as through a capillary with a diameter of 10 μ . Therefore, the opening of the shunts brought about reflexly all over the periphery in severe cold may protect the acral parts to some degree from tissue death (127, 128). On local or general heating the anastomoses are found to be open, but blood flows through the capillaries, too, in spite of the great amount of sidetracking of blood. Thereby heat dissipation becomes maximal (127, 128).

The Clarks (63, 64) (by using the ingenious technic of observing cutaneous circulation in the rabbit's ear through a built-in glass chamber) have demonstrated by direct observation that arteriovenous anastomoses behave in much the same way as do arteries and arterioles in respect to both contractile and dilatory stimuli but that the anastomoses are decidedly more active and show a greater tendency to independent action. When these authors subjected the ears of rabbits repeatedly, over a period of several months, to various types of irritation causing increased blood flow, such as heating, mechanical and chemical stimulation, they found that new arteriovenous anastomoses developed. When the blood flow was permitted to return to normal, they often reverted to the normal capillary status.

Richards (234) raised the question of whether a different number of arteriovenous anastomoses could be responsible for the fact that there are habitually cold- and warm-handed persons. He pointed out that persons who work long hours outdoors usually have cold and cyanosed hands, while indoor workers tend to be warm-handed.

Since the work of Chambers and Zweifach (299) it has become clear that arteriovenous shunts exist not only between arteries and veins but also between arterioles and venules and between metarterioles and adjacent venules. In the latter instances the shunts are only about 15–20 μ in diameter.

According to Zweifach (299), the capillary loops of the nail fold of man represent a direct connection between metarterioles and venules.

Masson was the first to state that physiologically occurring arteriovenous anastomoses may form the basis for the development of pathological "glomus tumors" (197).

V. NONNERVOUS VASCULAR REACTIONS

Since Lewis' fundamental work on cutaneous minute vessels (186), local vascular responses are differentiated as "direct" and "indirect" reactions. Direct reactions result from action directly on the vessel wall or, more specifically, on their contractile or expansible elements, without any intermediary mechanism. Indirect reactions are spoken of if the stimulus liberates a substance from neighboring cells which, in turn, acts on the vessel wall. With this definition, nervous reactions belong to the indirect responses because the nervous impulse acts through the liberation of a substance at the nerve ending.

Direct reactions are characterized by being confined exactly to the area of stimulation without any spread beyond it. Although it has been suggested that an impulse may be propagated from one contractile cell to the other (92), such propagation has not been proved and is most improbable (170). Indirect reactions, which spread slowly from the area of stimulation with a velocity of 1 mm. per minute or less, are usually caused by a liberated substance or, more generally, by the production of some metabolite. Widespread reactions with rapid, almost momentary, spread are usually results of nerve impulses.

A. CONSTRICTOR RESPONSES

There is a variety of stimuli which act directly on cutaneous minute vessels and cause contraction if the intensity of the stimulus is low, but dilatation if the intensity is high. The most commonly encountered stimuli of this kind are mechanical stimuli, usually studied on humans by stroking the skin. But the same effects can be elicited also by cold stimuli (186) and by galvanic current under the anode (141).

Usually, in studies on man the rounded smooth edge of a flat ruler or a tongue blade is drawn steadily but not roughly in a straight line across the skin. After 10–15 seconds the stroked area becomes distinctly paler than the surrounding skin ("dermographia alba"). The paleness is strictly confined to the area of stimulation. The reaction is fully developed in 1 minute and lasts for several, usually for 3–5, minutes (186). This reaction is due to contraction of muscular elements of minute vessels (metarterioles and precapillary sphincters). It is independent of arteriolar contraction (186). It is independent of the blood flow and blood pressure; it occurs also if the circulation is occluded. Moreover, it is independent of nervous impulses; it can be elicited in denervated areas as well as in skin with intact innervation. The stimulus actually is not pressure as exerted by the stroke but tension or stretching. Lewis (186) demonstrated this by showing that "if two fingers are placed an inch or so apart on the skin of a susceptible subject and the skin in between them is lightly stretched it is this stretched skin rather than that with which contact has occurred which subsequently pales." The contractile force exerted by the minute muscular vessels is capable of resisting a pressure of from 50 to 100 mm. Hg. Susceptibility to this reaction varies greatly with the individual. Attempts were made to work out a standardized quantitative method for measuring the degree of the reaction as an index of excitability of minute vessels (85). It was found that the white line is elicited with greater ease in young persons than in subjects over thirty (92). It is augmented by hypercapnia and weakened by anoxemia (85). Apparently, it is easier to elicit the "white line" in warm than in cold

skin (92), but the influence of skin temperature on the threshold is not conspicuous (85).

Local vasoconstriction following gentle stroking or stretching is accompanied in some subjects by pilomotion. The goose flesh is restricted to the area of stimulation and is independent of the nervous system (71), as is the white reaction. Though coupled in some instances, the two reactions are independent of each other. It depends on individual susceptibility whether vasoconstriction or pilomotion will prevail, or, in other words, which contractile elements, those of the minute vessels or the pilomotor muscles, are more responsive.

The effects of gentle stroking can be perfectly simulated by the injection of epinephrine into the skin. However, to establish this analogy, highly diluted epinephrine solutions must be used, such as 1:100,000 or higher. With such dilutions the responses to epinephrine are as fleeting as those to stroking. Higher concentrations of epinephrine, as they have been used in so-called "pharmacodynamic tests" of the skin, cause an unphysiological long-lasting spastic state of both minute vessels and pilomotor muscles, and it is hardly probable that individual differences could be detected with such coarse methods. Also it should be stated emphatically that testing of the contractility of cutaneous vessels by stroking or by epinephrine gives information exclusively on the contractile excitability of metarterioles and capillary sphincters and no information whatsoever on the excitability of constrictor nerve fibers or, worse than that, on the "psychosomatic" excitability of the individual (243).

In intense acute inflammatory processes the local vasoconstriction cannot be elicited either by stroking (284) or by local injection of epinephrine (240); or, at least, abnormally high intensities of stimulation are needed to provoke it. Apparently, the acute inflammatory process "paralyzes" the muscular elements of the maximally dilated precapillaries, making contraction impossible. A similar paralysis is present in the noninflamed skin reddened by cold (see p. 70).

On the other hand, in most of the subacute and chronic inflammatory processes the "white line" can be provoked with greater ease than in normal skin (284). Among dermatoses with conspicuous contractile excitability of the walls of cutaneous vessels, Whitfield (284) enumerates pityriasis rubra Hebrae, seborrheic dermatitis, and "secondary flexural dermatitis."

If it is true that contractility is diminished in acute inflammations but is augmented in subacute and chronic inflammatory processes, it certainly would be desirable to have some explanation for this behavior. Experiments of Lewis (186) suggest some tentative interpretation. He found that, in contrast to minute vessels which have become unresponsive to vasoconstrictor stimuli at the site of injury in acute inflammation, such refractoriness to constrictor stimuli does not exist in the so-called "flare" surrounding the site of injury and deriving from dilatation of "strong arterioles." On the contrary, the blanching in the flare is unusually intense, as White described it in chronic dermatoses. Thus one may speculate that in acute inflammatory processes, such as follow mechanical, chemical, thermal, and actinic injuries and such infections as erysipelas, primary dilatation of the metarterioles and precapillary sphincters prevails (as in the "local red reaction"), while in subacute and chronic processes the reddening is due to a primary dilatation of strong arterioles (as in the "red flare"). This difference also would explain the different shades of red: bright or brilliant red in acute, dusky red in chronic, inflammations. In other words, in acute inflammation dilatation of the subpapillary plexus prevails, while in subacute and chronic inflammations dilatation of strong arterioles is the primary event. Thereby the red color deepens (see also p. 69).

Probably the most important pathological implication of the local vasoconstrictor reaction is its exaggeration in atopic individuals. In these subjects gentle pressure on the skin causes an unusually intense and long-lasting paleness, even on uninvolved

skin. The increased tendency of vasoconstriction in these persons was quantitatively followed by Eyster, Roth, and Kierland (107) when they found that the skin of atopic patients showed a swifter rate of cooling than normal in a cool environment, delayed rate of warming in a warm environment, and exaggerated responses in the cold pressor test.

minutes to an hour (Fig. 10). This blanching can be counteracted by local atropinization. Lobitz' tentative interpretation has been that the paradoxical response may be analogous to that found by Burn (48): if acetylcholine is leached out of isolated rabbit-ear preparations by long-continued perfusion, the addition of acetylcholine to such a preparation causes vasoconstriction initially. An

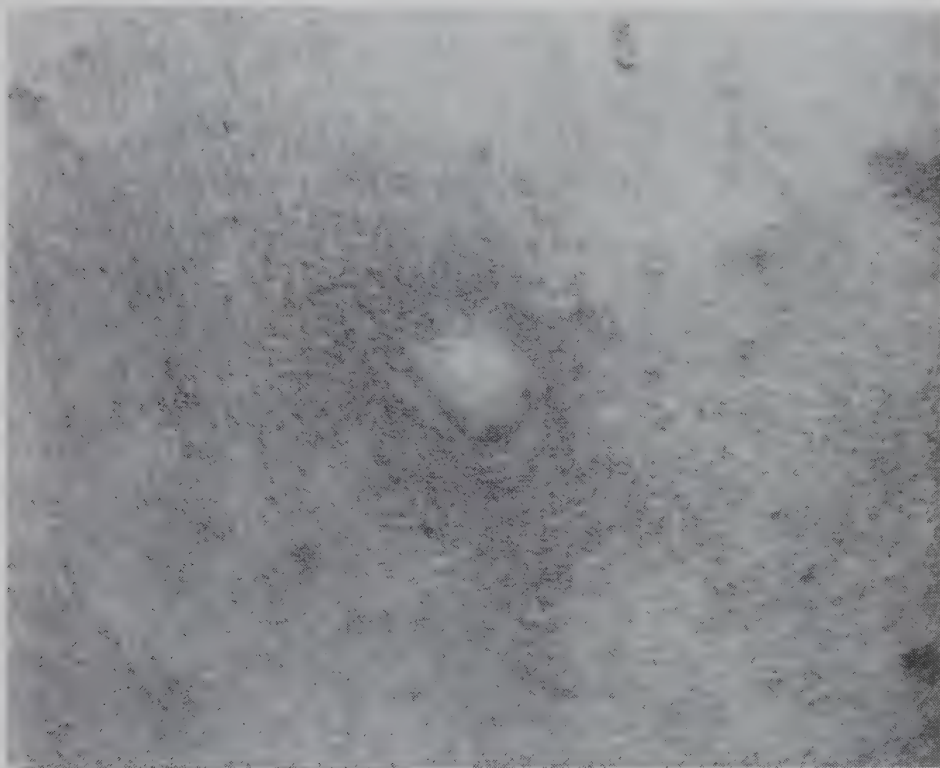


FIG. 10.—“Delayed blanch” in an atopic subject following injection of acetylcholine. From Lobitz and Campbell (191). (Reproduced by permission of Dr. Lobitz and the American Medical Association.)

Most recently, Lobitz, Jr., and Campbell (191) discovered a novel and paradoxical vascular reaction in these individuals. They confirmed the increased contractility of minute vessels in response to stroking but found no differences from normal in responsiveness to epinephrine. However, a marked abnormality occurred on injection of acetylcholine. While in normals the direct local effect of this agent on minute vessels consists of a slowly developing, long-lasting vasodilatation, in atopic subjects it causes a delayed, slowly spreading vasoconstriction (“delayed blanch”), which appears 3–5 minutes after injection and lasts from 15

alternative explanation would be that in atopic individuals excess acetylcholine leads to liberation of an excess amount of epinephrine.²

2. L. Illig has demonstrated that the majority of neurodermatitis patients respond to the percutaneous application of nicotinic acid esters with vasoconstriction, as contrasted with the normal hyperemic reaction. Anemic reaction was seen in 33 out of 47 neurodermatitis patients, while none of 178 non-neurodermatic test subjects reacted in this way (L. Illig, *Die Reaktion der Haut des Neurodermitikers auf zwei nikotinsäureesterhaltige Reizstoffe*, *Dermat. Wchnschr.*, 126:753–63, 1952). Thus the paradoxical vasoconstrictor reaction to hyperemizing agents in neurodermatitis does not seem to be restricted to the response to acetylcholine.

B. DILATOR RESPONSES

If the mechanical stimulus is of greater intensity than that which elicits vasoconstriction, a red local reaction ensues. The "red line" ("dermographia rubra") which develops on intense linear stroking has a latent period of only 3–15 seconds and reaches its height in $\frac{1}{2}$ –1 minute. Its duration depends on the intensity of stimulation and reactivity of the vessels. After low-grade stimulation the red line may disappear in a few minutes. If the stimulus was strong, it may last for half an hour or longer. The line is bright red but later gradually assumes a bluish tinge. Similar local vasodilator impulses can be obtained on thermal, electrical, and chemical stimulation.

This reaction comes about by active dilatation of the smallest blood vessels having muscular elements (57, 92, 186). The reaction involves increased blood flow through all the minute vessels and a moderate increase in skin surface temperature.

Like the white line, the red line is confined to the area of stimulation and is independent of nervous impulses. It occurs with the same intensity in areas the nerves of which have degenerated as in areas with intact nerves. From its appearance, intensity, and duration no conclusions can be drawn concerning the nervous excitability of the individual. Sometimes a pale zone of vasoconstriction (and pilomotion) can be seen around the red line. Obviously, the central part of the stimulus is strong enough to elicit the red reaction, whereas on the periphery its intensity just suffices to cause constriction.

C. WHEEL FORMATION

Whealing represents circumscribed superficial edema of the skin as it develops in response to various chemical, mechanical, thermal, and actinic stimuli. It is always preceded by local reddening, as described in the preceding paragraph, and, like the red reaction, it is, at least for some time, sharply confined to the area of stimulation. Thus, by means of this edematous reaction, it is possible to "write on the skin" with sharply

outlined types if the stimuli are shaped in the form of letters (Fig. 11). One to 3 minutes after the wheal-producing stimulus is applied, the reddened area begins to swell, and it reaches its maximum height in 3–5 minutes, projecting 2–5 mm. above the surface. Usually, the elevation is flat on the top and smoothly rounded at the edges. Very soon after its full development the originally red wheal blanches, in most cases becoming paler than the surrounding skin. Before disappearing, its borders may become less sharply defined, and the wheal becomes slightly broader and flatter. A most characteristic feature is its limited duration. The average duration is 2 hours, and a true wheal hardly ever lasts longer than 4 hours. This description fits both experimentally produced and naturally occurring wheals. The latter, particularly, are often designated as "urticarial wheals."

Whealing is caused by leakage of blood plasma from the dilated minute vessels into the extracellular spaces of the papillary and subpapillary layers. The fluid causing the swelling contains up to 84 per cent of the plasma proteins but none of the cellular elements of the blood. The pallor is due to the pressure exerted by extravasated plasma on the minute vessels (186). Whealing requires increased blood flow. If stasis is produced by external pressure, wheal formation is impeded as soon as the external pressure comes close to the systolic blood pressure.

There are two distinct steps in the development of wheals: local vasodilatation and locally increased capillary permeability. Usually, the same stimulus causes local reddening, if applied in low intensities, and wheal formation above a certain intensity. This is the reason that the "erythematourticarial toxic eruptions" of dermatologists display two different types of lesions: non-elevated red patches and urticarial wheals.

Lewis (186) insisted that increased intracapillary pressure, distention of the capillary lumen, or reduction of pressure in the extracellular spaces does not suffice to account for wheal formation but that there must be a true (qualitative) change in permeability.

He found that considerable suction (negative pressure of 90 mm. Hg) applied to the red line did not cause wheal formation; positive pressure of 50 mm. Hg applied from the outside did not prevent it; and increase in the capillary pressure, produced by hindering venous backflow, did not promote it. In

given, fluid and dye were extravasated through the capillaries, but most of the fluid was reabsorbed at the venular end. After large doses or prolonged administration of histamine, the filtration became more intense, and the reabsorption of fluid was hindered by the high intravascular hydro-



FIG. 11.—“Writing” on skin in dermatographia elevata. *FU* = factitial urticaria

addition, the high protein content of the transudate also indicated that true increased permeability of the capillary walls and not simply a rise in filtration pressure was responsible for whealing.

In contrast, on the basis of experiments with dyes, Dekanski (82) found that wheal formation can be accounted for by raised intracapillary pressure and increased filtration. When small doses of histamine were

static pressure and by increased extravascular colloid osmotic pressure. Nonetheless, Lewis' evidence for true increase in permeability appears very convincing.

Chambers and Zweifach (60) discussed the difficulties in differentiating between the effects of a shift in hemodynamic relationships, of changes in functional permeability of the wall of capillaries, and of actual disruptive leakage of vessels. They themselves

found that histamine causes changes in capillary permeability only in concentrations which bring about actual endothelial damage, demonstrable by sticking of carbon particles, swelling of individual endothelia, etc. They emphasized that edema formation does not necessarily imply change in capillary permeability. Sympathectomy in itself, for instance, by causing vasodilatation and increased hydrostatic pressure in the capillary bed, greatly increases the tendency toward outward filtration without qualitatively changing the capillary wall.

In order to elicit wheal formation in the skin of normal subjects by mechanical means, the stimulus must be strong, such as energetic pricking with a pin or a repeated hard stroke. Only extremely intense mechanical stimuli, such as the lash of a whip, will invariably produce whealing in all normal individuals. Among thermal stimuli, only local application of extreme cold is certain to produce wheals in normals, in my experience. Lewis claimed that it can be regularly produced also by heat stimuli (186).

The easiest way to elicit wheal formation in normals is the introduction of urticariogenic chemicals into the skin. A great many materials are urticariogenic, among them weak acids and bases, commercial peptone preparations, alkaloids (e.g., morphine salts),³ materials from nettles, insects, etc. Since the advent of the fruitful theory that all urticariogenic agents act through liberation of histamine (or a histamine-like substance) (pp. 94 ff.), solutions of histamine salts have become the most widely used urticariogenic substances for experimental purposes. Histamine causes wheal formation in concentrations as low as from 1:1,000,000 to 1:2,000,000.

Pathologically susceptible individuals, suffering from so-called "factitial urticaria," develop wheals in response to mild stroking

or mild pressure. This "dermographia elevata" is the only pathological variety of "dermographism" in response to mechanical stimulation. White and red lines, also called "dermographia alba" and "dermographia rubra," as mentioned above, are physiological reactions. Like individuals whose skin is pathologically susceptible to mechanical stimulation, there are other persons who respond with whealing to moderate degrees of cold ("cold urticaria") or of heat ("heat urticaria"), to ultraviolet rays in the range between 2967 and 3341 Å, with a maximum at 3131 Å (34, 27), and in the range between 4000 and 5000 Å (35). All these stimuli elicit wheal formation in the pathologically susceptible individuals in extremely low intensities which do not cause any visible reaction in normals.

Whealing susceptibility to mechanical and cold stimuli can possibly be regarded as the pathological exaggeration of a physiological reaction. In the case of factitial urticaria it is sometimes difficult to draw the line between physiological and pathological responses. However, the spectral ranges of light mentioned above are not known to cause whealing in normals even if applied with extreme intensity, and in my experience the same is true for heat.

It also should be considered that in mechanical, actinic, and cold urticaria, cases in which passively transferable antibodies are demonstrable, the mechanism is possibly different from the corresponding physiological responses (pp. 97 ff.).

3. Recently direct evidence was presented for the histamine-liberating effect of morphine and other opium alkaloids. The skin can almost be depleted of histamine by these compounds, and the liberated histamine appears in the perfusion fluid (W. Feldberg and W. D. M. Paton, Release of histamine from skin and muscle in the cat by opium alkaloids and other histamine liberators, *J. Physiol.*, 114: 490-504, 1951).

VI. VASOCONSTRICTION OF NERVOUS ORIGIN

A. CENTRAL VASOCONSTRICTOR
IMPULSES

Stimulation of sympathetic fibers supplying the skin causes constriction of the cutaneous vessels in the area supplied by the stimulated fibers. Small arteries, arterioles, and, to a lesser degree, metarterioles, precapillary sphincters, and veins participate in this response. The vasoconstriction causes the skin to blanch and, if the state of constriction is maintained, to cool. The total water content of the skin decreases.

Conversely, if the sympathetic pathways are interrupted surgically or paralyzed otherwise, the small arteries and arterioles dilate. The dilatation causes a considerable increase in blood flow through the skin and a corresponding increase in skin temperature. Therefore, it must be assumed that normally the cutaneous small arteries and arterioles are under the influence of continuous efferent constrictor impulses which keep the vessels in a state of tonic contraction. Actually, it was shown by Adrian, Bronk, and Phillips (9) that efferent sympathetic fibers carry persistent electrical discharges which are chiefly concerned with vasoconstriction. These discharges fluctuate continually in an intermittent rhythmic manner. Plethysmographic studies of blood flow in fingers indicated that periodic constrictions of nervous origin occur every 30-60 seconds in all digits simultaneously (53, 49). It appears that the nervous control causes a continuously varying fluctuation between high and low values rather than an appropriate constant level of flow. The discharges are of central origin and seem to be an intrinsic property of the center, because they occur independently of any demonstrable stimulation. The autonomic controlling center of vasomotor tone is situated in the hypothalamus (234). The medullary vasomotor center studied by Ranson and Billingsley (225), which apparently controls the vascular tone in internal organs, does not seem to enter into the control of cutane-

ous vessels (234). The hypothalamic center functions autonomously, notwithstanding the destruction of higher parts of the brain.

Although man is unable to exert any voluntary influence over the tone of cutaneous vessels, the pallor caused by startle, fright, and other emotions makes it evident that efferent vasoconstrictor impulses may arise in the higher brain centers. The cortical center of emotional paling, as well as that of other cortical autonomic impulses, has been placed in the premotor area (234) (p. 174). Fibers originating there and connecting the cortex with the hypothalamus are regarded as neurons of the first order in the efferent vasoconstrictor pathway. Neurons of the second order run from the hypothalamus to the lateral horn of the gray matter in the spinal cord. Neurons of the third order arise from cells in the lateral horn, leave the cord through the ventral roots of the thoracic and lumbar segments, and reach the sympathetic trunk via the white rami communicantes. These myelinated fibers are the preganglionic fibers of the sympathetic system; they terminate in the sympathetic ganglia, their ramifying axons surrounding the sympathetic ganglion cells (Fig. 12). The postganglionic, nonmedullated fibers, constituting neurons of the fourth order, originate in sympathetic ganglion cells and leave the sympathetic trunk via the gray rami communicantes. They reach the periphery in the mixed peripheral nerves and end in the vessel walls with widespread ramifications of their axons. The distribution of the fibers is strictly unilateral. The fibers stop abruptly at the mid-line of the body, while the blood vessels themselves anastomose freely across the mid-line. Preganglionic fibers to the head and neck leave the cord by the first four thoracic ventral roots and pass to the superior cervical ganglion, where the postganglionic fibers arise.

Postganglionic vasoconstrictor fibers are adrenergic (76). Their impulses act by liberating nor-epinephrine at their endings,

which, in turn, effects the vascular contraction (p. 163).⁴

The paralytic vasodilatation of the skin after sympathectomy is not permanent because compensatory mechanisms gradually set in (133): (1) the intrinsic tone of vascular smooth muscles increases; (2) the smooth-

4. It was thought for a long time that two different substances are liberated at adrenergic nerve endings—sympathin E and sympathin I (55)—because some effects of sympathetic stimulation were found to differ from stimulation by epinephrine (18). But recent work has left little doubt that the stimulating mediating substance of adrenergic fibers is nor-epinephrine. Euler (103, 104), who isolated nor-epinephrine from sympathetic nerves, thinks that the assumption of the existence of sympathin I

muscle cells acquire an increased sensitivity to local vasoconstrictor stimuli, e.g., metabolites, cold, and epinephrine (87, 117); (3) in preganglionic sympathectomies the sympathetic ganglion cells remain intact and may respond to humoral stimuli, although their connections with the central nervous

has become unnecessary, while sympathin E is identical with nor-epinephrine. It was shown that nor-epinephrine is released also by the adrenal glands, together with epinephrine (45, 44, 281).

Ahlquist (11) distinguishes "alpha receptors" associated with most excitatory adrenergic functions and "beta receptors" associated with most inhibitory functions. Janowitz *et al.* (157) found that the human skin contains only alpha receptors.

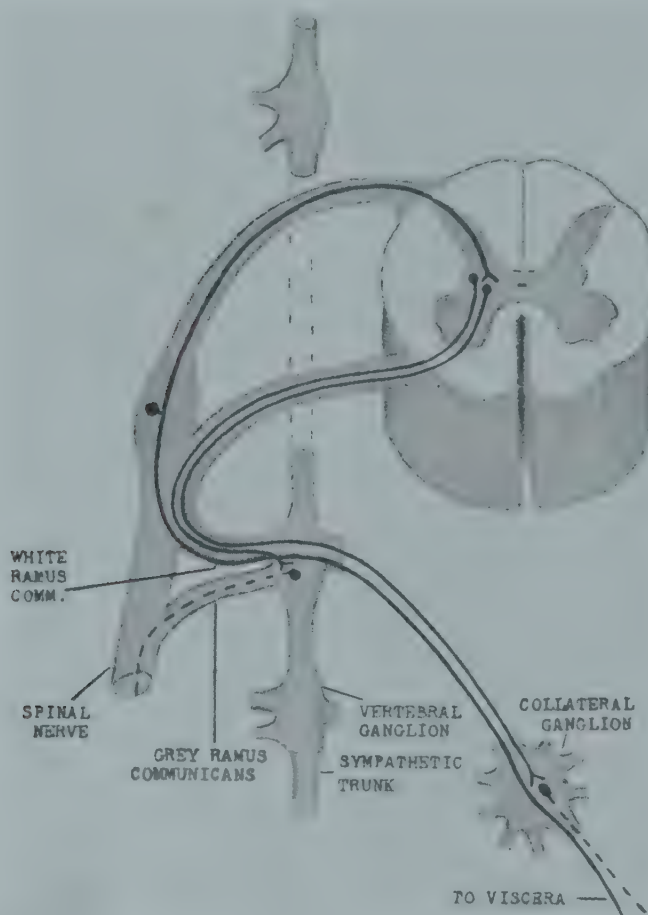


FIG. 12.—Diagram showing the connections of sympathetic fibers and of an afferent (sensory) visceral fiber. The preganglionic sympathetic fiber (*solid line*) emerges from the cord through the anterior root, passes the white ramus communicans, and ends in the vertebral ganglion of the sympathetic chain. The postganglionic fiber (*broken line*) originates from a sympathetic ganglion cell and joins the spinal nerve via the gray ramus communicans. The afferent (sensory) fiber has its cell body in the dorsal-root ganglion (*solid line on left side of diagram*). From here a central branch enters the cord through the posterior root, and a peripheral branch connects to the viscera. From C. H. Best and N. B. Taylor, *The physiological basis of medical practice* (New York: William & Wilkins Company, 1950).

system are interrupted; (4) sympathetic fibers have a remarkable power of regeneration and are able to bridge large gaps (16).

Vasodilator paralysis in limbs after sympathectomy is essentially restricted to the skin. It appears that circulation in muscles is primarily more influenced by local tissue metabolites, while the central sympathetic tone of the cutaneous vessels is as important in regulation as are local factors (279, 265).

B. VASOCONSTRICTION BY LONG REFLEX

Impulses arising in the periphery either from the vascular system itself or from other peripheral nerve elements may be carried to the central nervous system by afferent fibers and there relayed to efferent vasoconstrictor pathways, with the result of more or less generalized vasoconstriction of the cutaneous vessels. Such long vasoconstrictor reflexes are elicited by cooling of circumscribed parts of the skin, by noise, by cutaneous pain stimuli, by chemical or mechanical stimulation of internal organs, by forced deep inspiration, by inhalation of tobacco smoke and of carbon dioxide, etc. (178). Deep expiration is also effective (83). This type of reflex remains unimpaired in cases with lesions of the cerebral hemisphere. The level of the relay at which the afferent impulse is switched to efferent constrictor pathways is not satisfactorily located. Recent studies on paraplegic patients (119) with complete disruption of the cord have suggested that in some instances cutaneous vasoconstriction could be a purely spinal reflex. Probably, however, the afferent impulse most commonly reaches the hypothalamus and is transferred there to constrictor pathways.

The afferent pathways are obviously quite manifold. In the case of inspirational vasoconstriction the impulse probably originates from pressoreceptors in the right auricle and intrathoracic veins (83). Reflexes in response to cutaneous stimuli apparently arise in cutaneous sensory end-organs and ascend in sensory pathways. In reflex vaso-

constriction elicited by cold, at least two mechanisms are operating: (1) afferent impulses originate in cutaneous cold receptors of the parts immersed in cold water and ascend to the vasomotor centers; (2) cooled blood returning from the immersed part reaches and stimulates the thermosensitive centers in the hypothalamus. In addition, there is increased release of epinephrine and nor-epinephrine from the adrenal glands in this reflex. Temporary occlusion of the circulation does not interfere with the reflex, an indication that it is a purely nervous phenomenon, not influenced by passive hemodynamic changes. The reflex is abolished in skin areas whose sympathetic supply has been severed (88).

The cutaneous reflex vasoconstriction is most pronounced in the acral parts, primarily because of the presence of a great number of arteriovenous anastomoses which become tightly closed by the constrictor impulse. A simultaneous sudden decrease in blood flow in fingers, toes, and nostrils was observed by Burton (49). Because of the great reactivity of fingers and toes and also because of methodological advantages, the reflex vasoconstriction was studied in most instances in these areas. However, there can be little doubt that extensive skin areas participate in the reflex, because it often leads to a measurable increase in the mean blood pressure (287). Work with the photoelectric plethysmograph also indicates the more or less general character of the vasoconstrictor reflex (84). However, the cutaneous vessels of the head participate only weakly in reflex vasoconstriction or may even dilate at the same time that digital skin vessels contract vigorously (p. 75) (84).

On the whole, the skin is a highly responsive, if not the most responsive, organ to vasoconstrictor stimuli. The difference in responsiveness is particularly marked if the skin is compared with skeletal muscle. Stimuli producing vasoconstriction in the skin have no such effects on the blood flow in the muscles and may even increase it (130). This is analogous to the effect of small doses of epinephrine, which cause constrictions of

cutaneous blood vessels but increase the flow through muscles. The difference between skin and muscle reactivity is due partly to the fact that muscles contain sympathetic vasodilator fibers (47), whereas in the skin such fibers have not been conclusively demonstrated (see below). Cutaneous vessels respond to both sympathetic stimulation and epinephrine by vasoconstriction exclusively (195, 125). Slow infusion of epinephrine causes a measurable increase in oxygen consumption and in body temperature. This effect comes about by cutaneous vasoconstriction, which reduces heat dissipation and thereby causes a rise in body temperature and consequently in the metabolic rate. That this assumption is correct was shown by Witcher and Griffith (283). They demonstrated that after removal of the skin from the whole body surface (except from the head, tail, and feet)

epinephrine is no longer effective in this regard (283).

C. VASOCONSTRICTION BY LOCAL REFLEX MECHANISM

Whether constrictor axon-reflex mechanisms (p. 93) operate within the skin is not certain. In frog skin Krogh (170) observed that if an artery is pricked, there is capillary contraction in the neighborhood which is dependent on intact sympathetic supply. In man, Coon and Rothman (71) observed the rapid development of pale spots in areas surrounding the site of intradermal injection of nicotine sulfate 1:10,000. Such injections, however, are known to stimulate the adrenergic pilomotor nerves (p. 170), and possibly the vasoconstriction observed was caused by liberation of excess nor-epinephrine, which might have induced vasoconstriction without the interference of nerve impulses.

VII. VASODILATATION OF NERVOUS ORIGIN

A. THE PROBLEM OF SYMPATHETIC VASODILATOR FIBERS TO THE SKIN

Three types of vasodilator fibers are known to occur in mammals, viz.: (1) parasympathetic fibers, originating in the cranial and sacral divisions of the central nervous system; (2) sympathetic vasodilator fibers; and (3) dorsal-root or "antidromic" vasodilator fibers. In addition, neurogenic vasodilatation results from vasoconstrictor paralysis with decreased vasoconstrictor tone (p. 85). Parasympathetic (cholinergic) vasodilator fibers, such as those innervating the salivary glands and the penis, are not known to occur in the skin.

The existence of vasodilator fibers which would reach the skin along the same sympathetic pathways as the vasoconstrictor fibers has been postulated on the basis of indirect evidence. One important observation has been that heating the body produces a greater blood flow in a normal extremity than in an extremity which has been sympathectomized (190). Therefore, it was

concluded that sympathectomy must have disrupted some vasodilator fibers; if reflex vasodilatation following heating were due only to sympathetic paralysis, the reaction should be equal on both sides. Warren *et al.* (279), however, pointed out that such an experiment would be conclusive only if it were carried out immediately after sympathectomy, because later there is a compensatory increase in vascular tone on the sympathectomized side (p. 86) which greatly complicates the situation. The same difficulty arises in interpreting the observation that the sympathectomized limb fails to flush on local heating (153).

If in the area of flushed heated skin a cutaneous nerve block is set, the skin pales (129). This phenomenon, too, was interpreted as evidence for the existence of vasodilator sympathetic fibers to the skin: seemingly, by paralysis of the mixed nerve, active vasodilatation is prevented. However, there was reason to doubt the validity of these experiments (279).

Experiments of Warren *et al.* (279) indicate that there are no vasodilator sympathetic fibers in the skin. These authors infiltrated sympathetic ganglia with procaine and found that the sympathetic paralysis thus produced in the hands led to as complete vasodilatation as did local heating or general heating. Sarnoff and Simeone (252), studying indirect vasodilatation in the skin by heating other parts, also came to the conclusion that this reaction is due exclusively to central inhibition of vasoconstrictor impulses and not to active vasodilatation. Thus the existence of vasodilator sympathetic fibers in the skin remains at least doubtful.⁵

B. ANTIDROMIC VASODILATATION

From the dermatological point of view, probably the most important vasodilator impulses to the skin are the so-called "antidromic impulses." If in the dog or cat dorsal roots are sectioned and the peripheral stumps stimulated, the skin of the corresponding dermatome segment becomes flushed by active arteriolar vasodilatation. This phenomenon was first observed by Stricker in 1876 (268) and was later studied in great detail by Bayliss (24, 25, 26). He found that the fibers causing vasodilatation did not degenerate after section of the dorsal roots if the section was made proximal to the dorsal ganglion, but they did degenerate if the dorsal ganglia were removed. He concluded that the nutrient cells of the vasodilator fibers were located in the dorsal ganglia. Bayliss (25, 26, 27) and Langley (180, 181) demonstrated that only cutaneous blood vessels were involved in this phenomenon. In man, O. Foerster (113) obtained cutaneous vasodilatation in segmentary distribution by stimulation of dorsal roots at various levels of the cord. It was

thought probable that vasodilator fibers, analogous to those in dorsal roots, also exist in cranial nerves, particularly in the trigeminal nerve which carries the impulses to the face and tongue. Electrical, thermal, and mechanical stimulations of the dorsal roots are equally effective in eliciting cutaneous vasodilatation.

It appeared that the dorsal roots convey not only afferent (sensory) impulses, as postulated by the Bell-Magendie law, but also efferent (vasodilator) impulses. Bayliss assumed that the centrifugal vasodilator impulses run in the same fibers as those which mediate the centripetal sensory impulses, and he termed the vasodilator impulses "antidromic," because, apparently, they were running in a direction opposite to that in which sensory impulses travel.

Barron and Matthews (20, 21) demonstrated electrophysiologically as well as by histological control of degeneration experiments that, beyond doubt, all vasodilator fibers have their nutrient cell stations in the dorsal-root ganglia, just as sensory fibers do. They found that a large fraction of the impulses which end with cutaneous vasodilatation are reflexes; they start in a peripheral sense organ and run through the dorsal root to the cord. Here the fibers carrying the impulse pass a long or short distance up or down the cord and leave it again by a dorsal rootlet, which might be far from the rootlet containing the entrant fibers. They also found that as high a proportion as 40 per cent of dorsal-root fibers can carry efferent impulses. At present it is still not decided whether the *same* fibers carry centripetal (sensory) and centrifugal (vasodilator) impulses or whether they are different fibers running together and having (at least in the periphery) the same relay station.

Lewis assumed that the antidromic fibers are distinct from sensory fibers when he developed his theory of a "nocifensor" nervous system (187) and when he postulated that this system is responsible for antidromic and axonal vasodilatation (p. 91) and for the spread of hyperalgesia (p. 131) but not for pain or other cutaneous sensa-

5. In recent experiments, Grant *et al.* presented evidence for the existence of sympathetic cholinergic vasodilator fibers in the central artery of the rabbit's ear (J. Armin, R. T. Grant, R. H. S. Thompson, and A. Tickner, An explanation for the heightened vascular reactivity of the denervated rabbit's ear, *J. Physiol.*, 121:603-22, 1953).

tions. This theory of a special nervous system serving defense reactions against skin injuries has not gained acceptance (274, 278) either anatomically or physiologically. But, irrespective of the theory as a whole, the possibility that sensory and vasodilator posterior-root fibers are separate has not been disproved. The simple fact that moderately intense pain stimuli do not lead to vasodilatation and, conversely, that some stimuli may elicit vasodilatation without causing pain seems to support the idea that there are two kinds of posterior-root fibers.

The antidromic vasodilatation, as compared with other vasomotor responses, is a delayed and prolonged response to a brief stimulus. The flush gradually increases to a maximal intensity over a period of about 20–40 seconds and then declines over a period of 5–10 minutes at an imperceptible rate (186). Because of this peculiar sluggishness, it was assumed that the nerve impulses act through the liberation of metabolites (181); and indirect evidence was presented by Lewis and Marvin (189) that this is actually the case. Dale and Gaddum (77) suggested in 1930 that the liberated substance could be acetylcholine, which dilates the arterioles primarily. In 1936 Wybauw (290, 291, 292, 293) actually demonstrated that antidromic vasodilatation is associated with the liberation of acetylcholine in the periphery. He identified acetylcholine in the perfusion fluid returning from a limb in which antidromic vasodilatation had been induced. This, together with the demonstration of liberation of acetylcholine in axonal vasodilatation in man (244), leaves little doubt that antidromic vasodilatation is a cholinergic impulse, at least primarily. The difficulty in accepting acetylcholine as the only mediator has been the relatively long duration of the vasodilatation, because acetylcholine, once liberated, is rapidly decomposed by the cholinesterases of the tissue. Lewis (186) suggested that, although the nerve fibers in question are cholinergic, the vasodilatation comes about in an indirect way: acetylcholine is released and acts upon cells in the skin, which, in turn, re-

lease the more stable histamine (or H-substance) which is responsible for the vasodilatation. It is hard to accept Lewis' interpretation, because the antidromic flush never wheals or itches. A much simpler explanation for the long duration of a cholinergic effect would be the assumption that the short nerve stimulation is followed by a series of afterdischarges.

However, Lewis' theory has gained support by the work of Kwiatowski (172), who demonstrated a marked increase in histamine content in the returning venous blood in the limbs of cats after antidromic vasodilatation. Kwiatowski even found histamine in the nerves themselves, with the highest concentrations in the distal parts of sensory nerves of the skin and generally "in all nerves which possess the property of antidromic vasodilatation." Therefore, in addition to the theory of indirect liberation of histamine by cholinergic impulses, Kwiatowski also considered the possibility of the existence of "histaminergic" fibers as possibly responsible for antidromic vasodilatation. That histamine within the nerve has some functional significance is indicated by the fact that it disappears when the nerve has degenerated (172). Von Euler (105), who also demonstrated the presence of histamine in nerve substance, came to the same conclusion. He stated that the great differences in histamine content of different nerves suggest some specific action of this "ergon," correlated to the function of the nerve, such as is known for acetylcholine and "sympathin." In favor of the histamine theory of vasodilatation it also can be said that facial flush can be elicited in man (137, 280, 156), by intravenous injections of carefully measured amounts of histamine, acting directly on the minute vessel walls.

The findings of Hellauer and Umrath (142, 275) indicate that possibly neither acetylcholine nor histamine but a third substance is the mediator of antidromic vasodilatation. This substance was extracted from dorsal roots and caused a slowly developing deep flush when injected into the normal or denervated rabbit ear. It was con-

clusively shown that this substance is different from acetylcholine and histamine.

While there is general agreement that axonal (local reflex) vasodilatation (see below) is due to impulses of the dorsal-root fiber system, it is not known whether or not physiologically purely efferent central nervous impulses, passing through posterior roots, arrive in the skin and cause vasodilatation. It would be quite important to know whether emotional blushing in man is or could be an antidromic impulse. The question has been dealt with rather cautiously by Lewis (186) but essentially has remained unanswered. In its development, emotional blushing is certainly similar to antidromic vasodilatation: it reaches its maximal intensity and extension in about $\frac{1}{2}$ minute and persists for about 10 minutes.

I have dealt with the phenomenon of antidromic vasodilatation in some detail because our present knowledge suggests that this type of vasodilatation may be of great significance in the life-processes of the skin under physiological, as well as pathological, conditions. First, it functions in response to local injury, and, second, it may come into play as a "psychosomatic" influence in inflammatory skin diseases. The antidromic impulse causes arteriolar dilatation, which increases the intensity of inflammatory processes, raises skin temperature, and decreases sensory thresholds (p. 136). Like emotional embarrassment, emotions of apprehension or resentment, so common in patients with itching skin diseases, may also cause arteriolar vasodilatation and exacerbation of the pathological process, possibly far beyond the areas of common blushing through central antidromic impulses.

Practical methods of preventing or counteracting antidromic vasodilatation have not been developed. If Lewis' and Kwiatkowski's assumption is correct, antihistaminics should be effective. They certainly are not effective in blocking axonal vasodilatation (193), an impulse which runs in the same pathway as antidromic impulses (see below). If the chief liberated metabolite is acetylcholine, its neutralization is a more

difficult problem. Atropine, in my experience, apparently does not block the vasodilator effect of acetylcholine, and I assume that this is because the effect on cutaneous blood vessels is not a "muscarinic" but a "nicotinic" action of acetylcholine (p. 169). However, in unpublished experiments, Dr. Lorincz (193) in our laboratory was unable to prevent axonal vasodilatation by high doses of nicotine (p. 169). Nor was he able to influence vasodilatation in man by the administration of the newer curare-like "anti-nicotinic" drugs.⁶ Recently, Lobitz and Campbell (191) found that some vascular effects of acetylcholine in the human skin can be blocked by atropine (p. 81). Thus it is obvious that further research in this field is highly desirable.

C. AXON-REFLEX VASODILATATION

If the stroke stimulus by which a "red line" is produced in normals is unusually strong or if the stroke is carried out on a subject with factitial urticaria, the originally sharply defined red line, after a few seconds, suddenly spreads into the surrounding skin. The flush or flare around the red line usually has a diameter of 2-3 cm. but exceptionally can be as large as 10 cm. In any case it is always much more extended than the local reaction. On the extremities the shape of the flare is usually roughly oval if the stroke has been made in the long axis of the extremity (186). Its edges are always irregular and poorly defined.

The flare develops later and disappears earlier than the local reddening. It does not become cyanotic but preserves its arterial tint until it disappears. The intensity of the color, however, is not uniform. Before its disappearance it may become quite mottled. The surface temperature on the flare is higher than in the area of local reaction, in-

6. While we were unable to counteract axonal vasodilatation by atropine in man, Armin and Grant have recently shown that in the rabbit's ear the vasodilator effect of acetylcholine can be abolished by small doses of atropine (J. Armin and R. T. Grant, The artery of the denervated rabbit's ear as a sensitive pharmacological test object, *J. Physiol.*, 121:593-602, 1953).

dicating greater arterial flow in the flare. While the local reaction comes about by relaxation of muscular elements of the smallest vessels, the flare originates from dilatation of arterioles (208, 72). These are the "arched" arterioles of Lewis (186), which supply from below the subpapillary arterial plexus and all of which supply areas as wide as 3-4 mm. in diameter. Their dilatation causes the smaller vessels to dilate passively, so that reddening of the surface ensues.

The red flare is the result of a nervous impulse. It fails to appear in skin areas the nerves of which have degenerated. Original-

for such mechanism operating in the red flare is given in Figure 13.

1. In skin areas anesthetized by local infiltration with anesthetics, whealing stimuli will elicit local reddening and wheal formation but no red flare. The reason for the absence of the flare is that the ramifying axon system has been made unresponsive to the injurious stimulus by the local anesthetic.

2. In skin areas anesthetized by block anesthesia of the supplying nerve trunk, the red flare develops with the same intensity and extent as in control areas, in spite of the prevailing complete sensory anesthesia. The

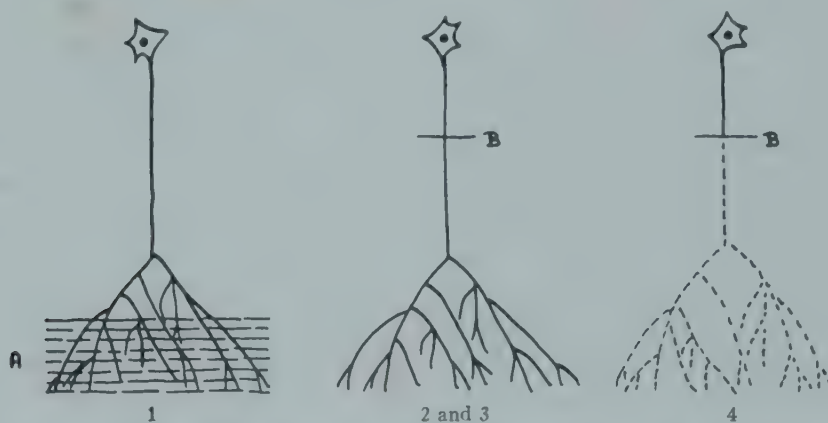


FIG. 13. 1, Local infiltration anesthesia at A causes rami axons to be nonresponsive. 2 and 3, block anesthesia or fresh nerve injury at B leaves the axon at the point of ramification and the rami intact. 4, an old injury at B, severing connection with nutrient center, has caused degeneration of fiber now unable to carry impulses.

ly, it was thought that development of the flare depends on a spinal reflex. The work of Bruce (43), Bardy (17), Breslauer (40), and mainly Lewis and Grant (188) has clearly established the fact that the red flare is the result not of a spinal, but of a local, reflex mechanism. This form of reflex, first called "axon reflex" by Langley (182), has no interpolated nerve cell in its path. The impulse caused by the injury travels centripetally through one or several stimulated branches of a ramified axon to the highest point of ramification and from there runs centrifugally over all branches stimulating the total area supplied by all branches. It is due to a considerable extension of the ramifications that the flare spreads far beyond the area of the original stimulus. The evidence

reason for the appearance of the red flare is that although the block interrupted the connection between periphery and center, it left the axon at the highest point of ramification and all the rami intact.

3. In skin areas the supplying nerve trunk of which has been recently interrupted by injury, so that the area has become anesthetic, the red flare again appears as it does in normal skin, the reasons being the same as in case 2.

4. In skin areas in which the supplying nerve trunk has been destroyed and time enough has elapsed for its degeneration, the red flare does not develop. The reason for its absence is that the peripheral end of the axon has been separated from its nutrient center and sufficient time has elapsed for the

peripheral part of the axon to degenerate and to become unresponsive to stimulation by the injury. Local reddening and local wheal formation remain normal in such areas. This behavior of the red flare is independent of the type of primary stimulation. It behaves in the same manner in response to mechanical, electrical, thermal, or chemical stimuli which normally produce a red flare.

The fibers carrying this axon-reflex impulse have their nutrient center in the dorsal ganglia. They were regarded by many as "sensory" fibers. Certainly, areas of analgesia and areas in which the red flare fails to appear coincide precisely, and this indicates the same pathway for the two functions. The possibility, however, that there are two sets of nonidentical posterior-root fibers has not been excluded, as discussed in the preceding section.

If conclusions by analogy with sympathetic axon reflexes are permitted, one may assume that the highest point of ramification of the posterior-root axon is in the mid-corium (p. 170).

Axon-reflex flare elicited from the periphery and antidromic vasodilatation elicited from ganglia or nerve trunks appear to be identical. Both operate only in the skin and possibly in adjacent mucous membranes; in internal organs neither antidromic vasodilatation nor axon-reflex flare can be elicited. Both have a latent period of 20–40 seconds; with short-term stimulation, both last 5–10 minutes and both behave as if they came about by the liberation of a vasodilator substance (189, 186).

It should be pointed out that the red flare is completely independent of sympathetic nerve supply. The reaction is seen to occur in a normal fashion after sympathectomies (165).

The nature of the mediating substance in the axon-reflex flare is as problematic as that of antidromic vasodilatation. Dale (75) suggested that the arteriolar dilatation is brought about by liberation of acetylcholine, and Rothman and Coon (244) actually demonstrated acetylcholine in liquid obtained by scarification of histamine wheals

in man when the decomposition of acetylcholine was prevented by local application of physostigmine. Still the intervention of histamine and/or of the sensory substance of Umrath and Hellauer (275) has not been excluded.

There has been anatomical evidence for collateral branches of "sensory" fibers supplying arteriolar walls and for such collateral paths extending for distances as long as 1 inch (289). It has been implied that in such an arc the afferent branch is "sensory," coming from a sensory receptor organ, while the different efferent branches lead to arterioles. Experience with sympathetic (pilo-motor and sweat) axon reflexes (pp. 168 ff.) suggests, however, that in the red-flare axon reflex, too, the impulse may run centripetally as well as centrifugally in all ramifications of the same axon and that all the branches are physiologically identical. The highest point of ramification apparently has some autonomous relay function comparable to that of ganglion cells (p. 171), with the double function of receiving the afferent impulse and then discharging it in the opposite direction over all rami.

It should be kept in mind that a portion of the hyperemia in any cutaneous inflammatory process could be due to axonal vasodilatation. The original experiments of Bruce (43) indicated that the inflammation itself has a milder course in denervated areas than elsewhere. The axon-reflex flare possibly also involves the central area of direct injury.

A surprising pathological finding has been recorded by Eyster, Roth, and Kierland (107). Their patients with atopic dermatitis responded to intradermal injection of histamine with normal whealing, but no red flare developed around the wheal in these subjects. Previously, Norrind (215) had noted that in such patients no "halo" developed around positive protein-allergen whealing skin tests. These findings, of course, fit well the observation of Lobitz (191) on vasoconstrictor responses to acetylcholine in such patients. If atopic individuals respond to acetylcholine with vasoconstriction, no cholinergic vasodilatation can be expected.

VIII. THE TRIPLE RESPONSE OF LEWIS AND THE HISTAMINE THEORY

The whealing reaction is composed of three component parts: (1) local vasodilatation of the minute vessels, causing local reddening;⁷ (2) local increased vascular permeability, causing local wheal formation; and (3) a vasodilator axon-reflex impulse with arteriolar dilatation, causing the red flare. This is Lewis' "triple response" of the skin to injury.

It has been the fruitful concept of Lewis (186) that this reaction, which appears quite uniformly in response to a great many and most heterogeneous physical and chemical urticariogenic stimuli, is so uniform because all the stimuli eliciting this response act not directly on vascular and nervous elements but by liberating a diffusible substance from the injured cells, which, in turn, stimulates the vascular and nervous elements when it reaches them by diffusion.

Indirect evidence for the humoral mechanism of the triple response was brought forward by Lewis and his co-workers in a brilliant series of experiments (186). One of the most impressive pieces of evidence was the behavior of the triple response if the stimulus was applied to the skin after the circulation had been arrested previously by external compression. The wheal does not develop as long as the occlusion lasts, because the external pressure hinders the outpouring of plasma through the injured vessels. However, after release of the occlusion, the triple response immediately appears and lasts exactly as long as it lasted when the circulation had not been arrested and when the wheal had formed right after stimulation. The delay by circulatory arrest can hardly be interpreted in any other way but as interference with some humoral mechanism. The released substance is retained as long as there is no blood flow to carry it away. Once the circulation is restored, the

removal of the substance takes the same length of time as it took when the circulation had not been interfered with.

Lewis assumed that the substance in question is histamine, which is known to occur in epidermal cells and which might be easily liberated if epidermal cells are injured.⁸ Furthermore, histamine is the only physiologically occurring substance which elicits the triple response. Among all known chemical compounds causing triple response, histamine is the only one which causes it in such extremely low concentrations as 1:1,000,000 to 1:2,000,000, a concentration which easily might be present in the tissue fluid after cellular damage. The triple response elicited by histamine behaves in all minute details and under all tested artificial conditions in exactly the same way as triple responses elicited otherwise and occurring under natural conditions (Fig. 14). Finally, if the triple response is elicited over large surfaces, a fall in blood pressure and an increase in gastric acidity are noted, signs which can be best interpreted as being caused by an overflow of excess histamine into the general circulation. Among others, Horton *et al.* studied this phenomenon in cold urticaria (150) and Beal in solar urticaria (27).

The histamine theory postulates that the last link in the chain of events in the development of the triple response is always liberation of histamine. Morphine, for instance, is urticariogenic, because if it is injected into the skin it causes liberation of histamine (p. 84). Some natural urticariogenic agents, however, contained preformed histamine, and, of course, in such cases liberation of histamine from within the cells need not be assumed. For instance, the fluid of nettle hairs (*Urtica urens*) has a histamine con-

7. The vasodilatory action of histamine is, according to Zweifach (299), most conspicuous on the precapillary sphincter and metarteriole.

8. Recent evidence strongly suggests that the main, if not the only, sources of histamine release are the mast cells and not the epidermal cells (J. F. Riley and G. B. West, The presence of histamine in tissue mast cells, *J. Physiol.*, **120**:528-37, 1953).

centration of 1:1,000, far more than sufficient to elicit a triple response (102). Probably, the commercial peptone preparations also act by virtue of their histamine content.

In the majority of the cases, however, the source of histamine is endogenous. That histamine is actually liberated in the skin and then carried away by the blood stream or enzymatically decomposed can be con-

cluded from findings on the decrease of histamine content in the skin after the development of the triple response (219, 214). Lewis himself was never able to demonstrate actually *in loco* the liberation of histamine following injury. Therefore, he called the hypothetical material "H-substance," indicating that it is either histamine or a histamine-like substance. By now sufficient evi-

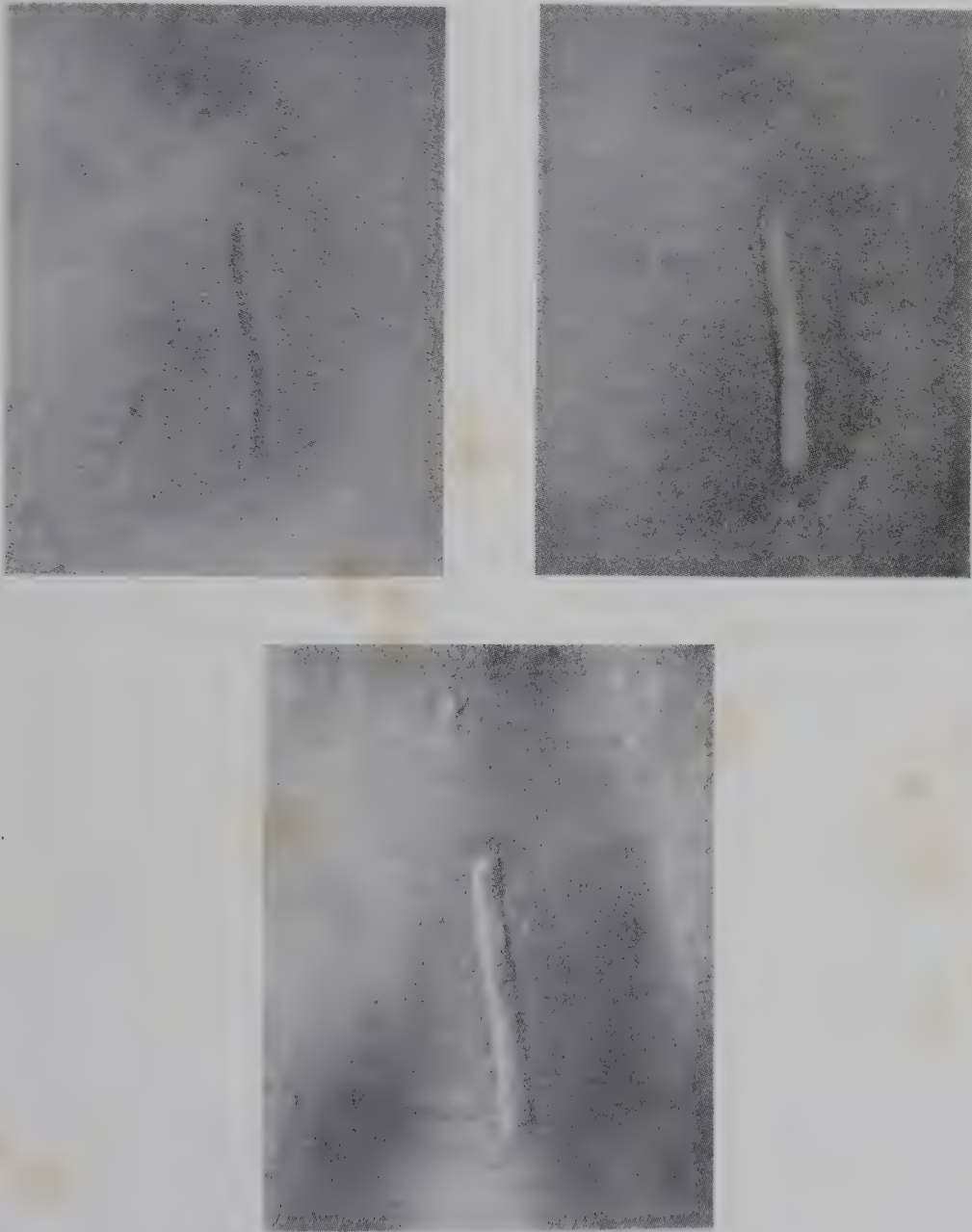
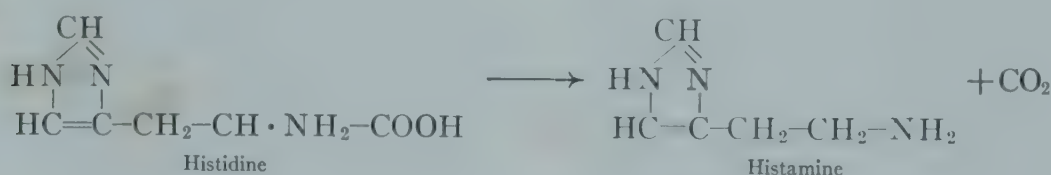


FIG. 14.— Development of the triple response to stroking and to histamine puncture (*top*). Photographs taken $1\frac{1}{2}$, $3\frac{1}{2}$, and 40 minutes after stimulation. From Lewis (186). (Reproduced by permission of Clinical Science.)

dence is available to show that the H-substance is actually histamine.

The histamine content of the normal skin is relatively high but very variable, according to species and individuals. Expressed in micrograms per gram wet weight of total skin, the determinations of Granroth and Nilzen (126) yielded the following values, expressed in micrograms per gram wet weight: guinea pig, 4.5–10.6; rabbit, 10.5 and 14.7; cat, 31.3 and 21.7; man, 8.5 and 7.7. These data match well with those of Tarras and Wahlberg (271) and with those of Emmelin (101). Gaté and associates (118) found higher values in human skin, namely, 10–22 γ /gm wet weight. All these analyses were carried out by the Barsoum-Gaddum technic as modified by Code (68). By this method histamine is unequivocally identified.



That such a mechanism may operate in actinic skin damage was assumed by Ellinger for a long time (96, 97). More recently two observations have emerged to support the view that histamine can be formed in the skin in response to injury. One is a considerable increase in the histamine content of burned skin as compared with that of normal skin (173, 81); the second deals with a compound which has no direct antihistaminic action but which acts pharmacologically by counteracting enzymatic histidine decarboxylation. This compound protects against anaphylactic shock, which is primarily a manifestation of histaminemia (206) (p. 97).

The expression "liberation" of histamine originally meant that intracellular histamine passed through cell membranes and acted in the extracellular space on the contractile elements of minute vessels. However, there is evidence that histamine is present in a bound form in the cell and that the first step

Increased histaminemia after extended urticarial reactions indicates that excess of histamine may be liberated in the skin, so that not all of it acts on cutaneous vessels but part of it is carried away by the blood stream. However, excess liberated histamine can also be destroyed in the skin enzymatically. Grauroth and Nilzen (126) found that skin extracts have the ability to destroy added histamine and that this effect is enzymatic. The "histaminolytic" effect is most pronounced in skin extracts of guinea pigs, quite appreciable in those of humans and rabbits, but absent in those of cats.

In addition to the release of preformed histamine from cells, the possibility of histamine formation in response to injury was also considered. Histamine can be formed enzymatically by the decarboxylation of histidine:

in its "liberation" is its release from a bound into a free state. In numerous experiments Dragstedt and his co-workers (91, 235) supported the theory that in the antigen-antibody reaction which ends with histamine liberation the first event is the activation or unmasking of a tissue protease; subsequently, the activated proteolytic enzyme splits off histamine from the tissue protein to which it was bound. Macintosh and Paton (194) found that a great number of organic bases (diamines and diamidines) liberate histamine from tissues; and they suggested a specific mechanism of histamine release: histamine may be displaced from its combination with tissue substances by chemically similar bases. It is interesting to note that such bases are likely to "sensitize" the skin and to elicit allergic reactions.⁹

Under pathological conditions histamine liberation occurs most frequently as the

9. For newer work on histamine liberators see nn. 3, 8, and 10 (pp. 84, 95, and 97).

end-result of antigen-antibody reactions. The urticarial type of hypersensitivity in man is based on histamine liberation in the skin. Generalized urticarial reactions in man, as they occur after ingestion of, inhalation of, or contact with foreign materials or after the action of physical agents, are based on a hypersensitivity mechanism. The organism has developed specific antibodies to the particular offending material, as can be shown in many cases by the passive transfer test of Prausnitz and Küstner.

The histamine theory of local (urticarial) allergic reactions was extended to systemic allergic reactions and to anaphylactic shock, the assumption being that the systemic signs of the shock are caused by an overflow of histamine, which is released in consequence of the hypersensitivity reactions of the tissues (110). Hypersensitivity reactions release histamine not only in the skin but also in many other tissues. For instance, if whole blood of sensitized rabbits is mixed with the sensitizing antigen *in vitro*, histamine is released from the cells to the blood plasma (160, 89). As early as 1910, Dale and his associates (18, 78, 79) suggested that histamine poisoning is a possible factor in anaphylactic shock. Since then, the histaminic mechanism of anaphylactic shock has been the subject of extended studies, with the result that, although some manifestations of the shock (such as decreased coagulability of blood, which is due to release of heparin [90], leukopenia, changes in sedimentation rate of red cells) are not directly due to histamine liberation, the chief event is indeed poisoning by histamine. Correspondingly, antihistaminics are life-saving in anaphylactic shock (109). The tremendous value of Dale's and Lewis' histamine theory became evident with the advent of these antihistaminic agents.

It has been a difficult problem to interpret the hypersensitivity mechanism of the so-called "physical allergies," in which a person rather suddenly acquires urticarial hypersensitivity to either rubbing, cold, heat, or ultraviolet light (p. 84). That antibodies are present in these conditions

has been proved by positive passive transfer tests (273, 8, 27). But what "antigens" these antibodies react with is difficult to fathom. The best current interpretation has been that of Sulzberger and Baer (270) and is as follows. Physical actions produce or liberate normal metabolites which do not cause any reactions in normals. However, hypersensitivity to any of these normal metabolites can be acquired in the same way as hypersensitivity is acquired to food ingredients or to drugs. In one instance this normal metabolite has been discovered: it is acetylcholine in heat urticaria. Patients with this affliction display a triple response to local injections of high dilutions of acetylcholine, and their trouble is that they respond in the same way to acetylcholine liberated in the skin by heat.

Interestingly, the same heat-sensitive patients suffer also from emotional urticaria, also called "cholinergic urticaria" (131, 149). This is the only disease whose psychosomatic mechanism is relatively well understood. Patients suffering from this disease are overwhelmingly young females with light complexion. They break out with generalized urticaria when they get excited and/or when they get warm. The disturbance in these subjects is not that they are exceedingly emotional; their nerve endings do not release more acetylcholine than normal or do not release it more readily, but the response of their skin to liberation of acetylcholine is pathologically increased. Acetylcholine not only causes physiological arteriolar dilatation but (probably through the mediation of histamine liberation) (187) also a full triple response. Cholinergic urticaria was regarded by the present writer as a pathological exaggeration of the blushing reaction (243).¹⁰

In the other physical allergies the "normal metabolite" formed under the action of the physical agent is not yet known. But all

10. Most recently it has been found that in patients with cholinergic urticaria blood cholinesterase levels are moderately reduced (J. K. Morgan, Observations on cholinogenic urticaria, *J. Invest. Dermat.*, 21:173-82, 1953).

have a histaminic mechanism, as is shown by their effective suppression with antihistaminics (248).

It should be kept in mind that in all pathological cases of urticarial hypersensitivity the basic pathological event is formation of antibodies and that histamine liberation is a result of antigen-antibody reactions. Triple response to histamine is a physiological and not a pathological reaction. It is not surprising that histamine intradermal tests cannot be used as a general indicator for allergy (108), because the

TABLE 2
DIFFERENCES IN ULTRAVIOLET REACTION
AND TRIPLE RESPONSE

	Ultraviolet Reaction	Triple Response
Latent period.	Several hours	Few seconds
Duration.	Several days	Few hours
Flare beyond area of stimulation.	Rare; few millime- ters; devel- ops slowly	Widespread, sudden
Pallor due to swelling.	Absent	Present
Swelling in subpapil- lary layer.	Present	Absent
Soreness.	Present	Absent
Itching.	Absent	Present
Blister formation. . . .	Present	Absent
Whealing.	Absent	Present
Effect of antihista- minics.	Absent	Present

resulting concentration of liberated histamine in hypersensitivity reactions is sufficient to cause whealing in anybody (186). For the same reason attempts at "desensitization to histamine" are based on a rather awkward concept. Critical review of the literature on "histamine desensitization" clearly shows that it is not possible to decrease appreciably the responsiveness of the skin to histamine.

While the histamine theory of urticarial and anaphylactic reactions has been most satisfactorily substantiated, Lewis' idea that all acute inflammatory processes of the skin are primarily based on histamine liberation could not be accepted. The evidence presented in favor of this view is indirect,

involved, and equivocal. The best example to illustrate the weakness of the "generalized" histamine theory is the case of the sunburn reaction. This reaction has a latent period of one to several hours. Subsequently, reddening develops which is sharply limited to the area of irradiation. Granted that in some cases, particularly when the irradiation dose is exceedingly high and/or the coverage has not been tight enough, a "diffusion flush" may be observed to creep into the nonexposed neighborhood, as shown in the illustration of Lewis (186). This spread, hardly ever more than a few millimeters, certainly cannot be compared with the widespread, suddenly appearing flare of the triple response. In addition to redness, proportionately to the dosage, swelling also develops in sunburn. This edema, however, although obviously based on increased capillary permeability, does not exert pressure in such a way as to press out blood from the dilated capillaries and cause paling. There is tenderness or soreness but no itching. If the irradiation is very intense, blistering ensues. The reaction lasts for several days and is followed by pigmentation and postinflammatory scaling. Thus there is nothing common between triple response and sunburn reaction, except that in both there is injury to the minute vessels (Table 2).

One of the least acceptable arguments of Lewis in favor of an identical mechanism in triple response and ultraviolet reaction is his reference to solar urticaria in hypersensitive individuals. He thought that "on this basis any remaining doubt as to the essential sameness of the chief mechanism underlying these acute responses, triple response and those to ultraviolet light, disappears." It is known today that sunburn reaction and urticarial reaction to ultraviolet rays have distinctly different action spectra (27), different pathomechanism (passive transferable antibodies in ultraviolet solar urticaria), and, while solar urticaria has a purely histaminic mechanism which is effectively suppressed by administration of antihistaminic drugs, nothing like that can be said of the sunburn reaction.

It is highly probable that any injurious stimulus applied to the skin which involves cellular damage causes liberation of histamine. For instance, in extensive thermal burns the histamine content of the blood is increased many fold (22, 69, 238). But obviously not enough histamine is liberated to cause whealing.

I believe that it should be most strongly emphasized that in the case of erythematous-edematous and blistering reactions the liberation of histamine, when it occurs at all, is not of primary significance, and nothing has been gained toward understanding these reactions by identifying them with the triple response, the purely histaminic nature of which has been amply proved. Antihistaminic drugs effectively suppress the triple response, but the blistering reactions are not measurably influenced by these drugs. No evidence has been presented so far which would disprove the classical morphological concept of four different types of cutaneous vascular reactions to injury.

The first is Lewis' triple response, characterized by local reddening, wheal formation, flare, and short duration. It has been regarded as the mildest and most superficial form of acute inflammation of the skin (272). The second is the burn reaction, seen in response to such insults as heat, cold, ultraviolet, ionizing radiations, and a great number of chemicals which in higher concentration cause frank necrosis. First-grade burn (erythema and edema), second-grade burn (blistering), and third-grade burn (central necrosis surrounded by inflammatory reaction) are all of long duration, and their extension beyond the injured area is rarely impressive. The axon-reflex flare within the inflamed area may aggravate the inflammation (p. 94), but only to a lesser degree.

Similarly, the third type of inflammatory process, the so-called "eczematous" type of dermatitis, in which intercellular edema of the epidermis with spongiotic vesicle formation (p. 94) develops prior to, or simultaneously with, the vascular reaction, has no similarity whatsoever to the triple response. It is strictly limited to the area of

injury (see sharp limitation of patch-test reactions); it lasts long and shows no signs of whealing.

Contrary to some preliminary observations (199), most observers agree that in the two latter groups of inflammatory reactions antihistaminic drugs have no preventive or curative action, or only a negligible one. This is particularly remarkable because eczematous reactions, as well as the triple response, are paradigmatically hypersensitivity reactions. It appears that there are histaminic and nonhistaminic hypersensitivity reactions in the skin. The basic difference between urticarial and eczematous hypersensitivity reactions was emphasized by the early great clinical investigator, Jadassohn (155). Experience with antihistaminics has strengthened the emphasis on these differences. In natural disease conditions there are hardly ever transitions between the urticarial and the eczematous type of inflammations. Such transitions should be expected, if the type of reaction depended only on the rate of histamine liberation and on its concentration in the tissue, as Lewis postulated. Efforts to demonstrate liberation of histamine in eczematous reactions, by estimating the histamine content in skin, blood, and urine, have led to inconsistent results (118, 219).

In a fourth type of inflammatory cutaneous reaction, the tuberculin-type hypersensitivity reaction (269), a considerable decrease in histamine content was demonstrated locally in the inflammatory papule (218). The development of the papule could be prevented by performing the reaction on top of locally injected antihistaminics (218). However, the fact that the reaction could not be prevented by systemic administration of antihistaminics (218) makes it rather doubtful that histamine release plays a decisive role.

In some diseases with prevailing blister formation, such as smallpox, chicken pox, herpes simplex, herpes zoster, the formation of red spots, persistent papules, vesicles, and necrosis are manifestations of different degrees of the same reaction. The triple re-

sponse does not fit anywhere into this series of events, and it is hard to accept the view of Lewis on the H-substance pathogenesis of herpes zoster (189).

In the discussion of the allegedly central significance of histamine in any kind of acute inflammatory reaction of the skin, mention should be made of the "leukotaxine" of Menkin (201, 202). This crystalline polypeptide isolated from inflammatory exudates greatly increases capillary permeability if injected intradermally and is thought to be the capillary-damaging agent in acute inflammatory processes. Although the controversial problem, with its voluminous literature, of whether leukotaxine acts via histamine release (82) or directly on blood vessels is still under discussion, it is certainly worth while to point out that one of the most characteristic features of acute inflammatory processes in the skin, namely,

accumulation of polymorphonuclear leukocytes, is not elicited by histamine, whereas the injection of leukotaxine simulates the picture of acute dermatitis rather closely. Menkin (202) reviewed all data which militate against the assumption that histamine is the primary agent in acute inflammation.

However, in any reaction connected with cutaneous cell destruction the skin rapidly becomes depleted of its histaminic content, because liberated histamine is almost immediately destroyed or carried away. The histamine content of the blood may rise.

The reactivity to histamine differs greatly according to species. Man is highly sensitive, and so are the guinea pig and dog. The rabbit's skin shows no reaction to histamine; in this animal the histamine content of tissues is low, that of the blood is high. The mouse and the rat show little sensitivity.

IX. VASCULAR RESPONSES TO THERMAL STIMULI

Vascular reactions subserving heat regulation are discussed in chapter 10. With increase in atmospheric temperature or in internal heat production, there is a generalized cutaneous vasodilatation brought about by long reflex nervous impulses and by warmed blood arriving at the hypothalamic thermoregulatory centers. Conversely, cooling or decreased heat production elicits general vasoconstriction in the skin. Although these reactions are more or less generalized, the acral parts play the most prominent role in them.

Remarkably, exercise, although it increases heat production, does not necessarily involve cutaneous vasodilator responses. Benedict and Parmenter (32) demonstrated that during and immediately after exercise there is a slight fall in skin temperature over the whole body surface, indicating

diminished blood flow, even if the subject feels subjectively warm. This fall in skin temperature is due to a diversion of blood from the surface to the striated muscles (see p. 248).

During sleep maximal peripheral vasodilatation was observed in both upper and lower limbs (162, 154). This phenomenon may contribute to the exacerbations of itching-scratching spells during the night.

Cutaneous arterioles also expand in response to local application of heat independently of the nervous system. However, as long as tissue damage is not elicited, the vasodilatation in response to local heating is less than the reflex vasodilatation on warming of the body as a whole (116, 285). Vasoconstriction in response to intense local cooling alternates with rhythmical periods of vasodilatation (pp. 261 ff.).

X. VASCULAR RESPONSES TO LOCALLY ACTING CHEMICAL AGENTS

A. METABOLITES AND HORMONES

The nature of locally formed metabolites regulating peripheral blood flow is still un-

certain. That there is a local control of small blood vessels by chemically acting vasodilators and vasoconstrictors is obvious

from experience in denervated areas (p. 86).

Among vasodilator substances, carbon dioxide and lactic acid were thought to be responsible for vasodilatation as it occurs in organ activity, but their concentration apparently does not account for the degree of vasodilatation (106).

1. *Acetylcholine*

Acetylcholine is probably one of the most important vasodilator metabolites in the skin, since it is the primary chemical mediator of antidromic and axonal dilator impulses. It acts on the arterioles, and therefore its systemic administration causes a drop in blood pressure. It also decreases the venous tone, thereby diminishing venous return and cardiac output (112). Intravenous injection of water-soluble salts of acetylcholine causes flushing mainly of the face, neck, and upper thorax and a general sensation of warmth (100, 56). Intra-arterial injection elicits flushing of the extremities also (100, 138). The greater reactivity of the blushing areas to vasodilator stimuli (p. 75) is borne out in these observations. Most effects of acetylcholine are potentiated by anticholinesterase drugs, which inhibit the cholinesterases of the tissues and thereby permit prolonged and intensified action of acetylcholine. The most important drugs with such action are physostigmine (eserine) and its derivatives and the group of alkyl and aryl phosphates (164). Conversely, most effects of acetylcholine are counteracted by atropine and other parasympathetic blocking agents, such as Banthine, Prantal, etc. Their blocking effect on cholinergic vasodilatation, however, is difficult to demonstrate (p. 91).

Therapeutic utilization of the vasodilator effect of acetylcholine is difficult because of its rapid decomposition in the tissues. However, acetylcholine derivatives (such as the methyl derivative, "Mecholyl"), which are more resistant to cholinesterases, have proved to be most effective vasodilators when applied locally, mainly by means of

electrophoresis of their water-soluble salts. This procedure significantly increases cutaneous blood flow (169, 70) and skin temperature (3). Its effect on blood flow is markedly greater than that of histamine (1).

2. *Nor-epinephrine and Epinephrine*

Among physiological constrictor metabolites, nor-epinephrine and epinephrine are the most important compounds. Nor-epinephrine is the mediator of sympathetic constrictor impulses. Both nor-epinephrine and epinephrine act on the muscular elements of all cutaneous vessels and are the most powerful constrictors of arterioles, metarterioles, venules, and veins of the skin. The lowest concentration of epinephrine causing just visible paling of the skin color in man is around 1:1,000,000. Vasoconstriction by adrenergic fibers, by epinephrine, and by nor-epinephrine is counteracted by the adrenergic blocking "sympathicolytic" drugs, such as the ergot alkaloids, β -haloalkylamines (with their main representative, Dibenamine), and the imidazolines (their main representative being Priscol). All these compounds block a step which is interposed between penetration of the cell by the exciting agent and the actual process of contraction (213). They are all more effective against responses to circulating sympathicomimetic agents than against responses to sympathetic nerve action (213).

Between epinephrine and nor-epinephrine, the former is more active in causing vasoconstriction in the perfused rabbit ear (196), although nor-epinephrine has the greater pressor effect (18, 195).

Whereas blood vessels of the muscles respond with dilatation to the injection of epinephrine (237, 130, 148, 171), such an effect does not occur in the skin. The dilator effect on vessels of the muscles is most easily demonstrated if the vasoconstrictor mechanism is paralyzed by administration of ergotoxine (so-called "adrenalin reversal") (74). Plethysmographic and venous outflow studies in the hind limbs of dogs after the removal of the skin have shown that in such

preparations epinephrine causes only vasodilatation, whereas in the intact leg the net effect is usually a reduction in volume because of the strong constriction of cutaneous vessels (151).

Cutaneous responsiveness to the constrictor effect of epinephrine is lost in reddened areas, where the minute vessels are dilated first, but is preserved in areas in which the reddening is due to primary dilatation of the arterioles with secondary passive dilatation of the smaller vessels (p. 80). For instance, there is no paling in sunburn erythema, but there is in the red flare around a wheal.

3. Pituitrin

Krogh (170) has thought that pituitrin (pitressin), the vasoconstrictor substance of extracts of the posterior lobe of the pituitary, may play a role in the maintenance of the physiological tone of minute vessels, because he calculated that barely twice the physiological concentration as it occurs in the blood (1:3,000,000) suffices to elicit pallor. Krogh (170) has emphasized that, in contrast to epinephrine, which acts with equal intensity on capillaries and arterioles, the pituitary substance is a specific capillary-contracting hormone. Its selective action on the smallest contractile vessels has been confirmed (249). This effect is not abolished by ergotoxine and is effective also in denervated areas (1). On systemic administration, pallor of the skin and mucous membranes becomes apparent after a few seconds. The face becomes involved first; then gradually the neck, ears, lips, and mucous membranes of the mouth become pale. Trunk and extremities react later (132). Generalized vasoconstriction induced by pitressin may not be accompanied by increased blood pressure, which suggests that arterioles are not involved in the reaction (135). The vasoconstrictor effect does not extend to the veins either; on the contrary, there seems to be a decrease in venous tone (264). A short, transient vasodilatation in the cutaneous vessels of dogs after intra-

venous injection of pitressin was investigated by Woodbury and Ahlquist (288).

4. Estrogens

The effect of estrogenic substances on peripheral circulation has been the subject of numerous studies, but the results have not been clear cut. It appears that natural estrogens and diethylstilbestrol do have a dilator effect on the capillaries of the skin but not on the arterioles. The rate of blood flow does not increase and the skin temperature is not elevated under the influence of these hormones (229, 7). The effect on minute vessels can be best demonstrated on the ear of ovariectomized rabbits (230). In man it cannot be elicited consistently (229).

It seems that in the menopause two types of dermovascular reaction occur (228, 232): one is a slow rise in the plethysmographic curve to a sustained plateau, while the second, "flush-type," response attains a maximum quickly, partially subsides quickly, but then persists at a lower but still elevated level. This second type seems to be related to menopausal flushes.

In view of the cyclic variations in the estrogen content of the blood, one might expect corresponding variations in the tone of cutaneous capillaries. Such variations were reported on the basis of capillary-microscopic observations (209). However, Reynolds and Di Palma (233), measuring the minimal necessary stimuli for vasoconstriction and threshold values for reactive hyperemia (p. 105), were unable to detect cyclic variations. Thus it remains undecided as to whether or not the frequently observed premenstrual exacerbation of inflammatory skin diseases is due to the dilator effect of estrogens. But there is a possibility that this is so, and, therefore, premenstrual exacerbations should not be taken as an indication that ovarian secretion plays a role in the causation of the skin disease in which premenstrual exacerbation occurs or as an indication that something is wrong with ovarian secretion in such cases. The fact that there

is a physiological vasodilatation in response to estrogens rather suggests that premenstrual exacerbation may occur in any inflammatory dermatosis, independent of its causation. This seems to be in agreement with clinical experience.

Clinical observations of Bean (28) led him to the assumption that the development of cutaneous arteriolar spiders and palmar erythema in liver diseases is promoted by a higher concentration of estrogens and possibly by anomalies in the metabolism of 17-ketosteroids, the decomposition of these hormones being disturbed in hepatic insufficiency. However, it is probably difficult to exclude the role of cutaneous venous stasis in liver diseases, as well as in pregnancy, when the tendency to vasodilatation is studied in these conditions. Sluggish capillary circulation in pregnancy with unusually frequent stasis and dilated venous limbs in the margin of the nail was first observed by Lennartz (184). Sludge formation was seen by Knisely (163), and such observations rather strongly suggest that the tendency toward the formation of telangiectases in pregnancy is primarily due to venous stasis.

Data on cyclic variations of cutaneous blood flow paralleling the menstrual cycle have been summarized by Abramson (1). The situation is far from being clear (136, 23, 233, 222, 41).

5. Male Sex Hormone

Reynolds *et al.* (231) observed marked lability and variability of cutaneous vascular tone with increased excitability of the minute vessels in castrate and eunuchoid males. This lability decreased after administration of testosterone propionate. To a lesser degree, progesterone had the same effect. In castrates, administration of testosterone propionate caused plethysmographically demonstrable vasodilatation, while normal males did not show such reaction.

That testosterone might have some vasodilator action is concluded from some clinical experience indicating its beneficial effect in the treatment of peripheral vascular dis-

eases (94). Vasodilatation after administration of testosterone propionate seems to be associated with an increased supply of oxygen-saturated blood to the surface capillaries (94). Schafer (253) found flushing of the skin after administration of large doses of testosterone propionate, an effect inhibited by estrogens. Conversely, spontaneous flushing in menopausal women was inhibited by administration of testosterone.

6. Adrenocortical Hormones

Desoxycorticosterone has no demonstrable effect on cutaneous capillaries (140). However, cortical extracts clearly prevent the increase in capillary permeability produced by leukotaxine (p. 100) (203, 115). These findings conform with the report that cortin is efficacious in the prevention and treatment of histamine shock. Combined treatment with sodium chloride and cortin is even more effective (221).

There can be little doubt that this effect of cortical extracts comes about by virtue of their 11-oxygenated steroid content. Cortisone and hydrocortisone are most potent sensitizers of the small blood vessels to the constrictor effect of nor-epinephrine and epinephrine. This effect was first demonstrated by Rachmiel Levine *et al.* (185) on the mesoappendix vessels of the rat. The administration of cortisone brings about a gradual increase of arteriolar tone in the normal skin, as shown by Ebert and Barclay (93) through direct observation of the rabbit ear chamber. This effect is even more impressive when inflammatory stimuli have caused vasodilatation. This vasodilatation is most effectively counteracted by cortisone, and at the same time the damage to the endothelium of arterioles and venules is greatly reduced (93).

There is general agreement that the dramatic therapeutic effects of ACTH, cortisone, and hydrocortisone in inflammatory cutaneous diseases are based primarily on their counteracting vasodilatation and subsequent phases of inflammatory reactions. How far the fundamental sensitization proc-

ess is also influenced, and not merely the reaction to which it leads, is still under discussion.

B. PHARMACOLOGICAL AGENTS

1. Vasoconstrictors

There is a great number of sympathicomimetic vasoconstrictor drugs which have some pharmacological properties in common with nor-epinephrine and epinephrine, e.g., ephedrine, Synephrine, Neosynephrine, Paredrinol, and Paredrine (259). They are less potent vasoconstrictors than epinephrine, but their effects last longer because they are not oxidized by the tissues as rapidly as nor-epinephrine and epinephrine. It seems that their effect on cutaneous vessels is weaker than on vessels of mucous membranes and internal organs. Thus, for instance, Neosynephrine and Paredrinol sulfate do not cause blanching of the skin (161, 254), even if given intravenously in doses which cause considerable increase in blood pressure. If Paredrinol has any vascular constrictive effect on the skin, it is slight as compared with its effect on splanchnic vessels (146).

A great number of reports indicate that tobacco smoking causes peripheral vasoconstriction. This view is based mainly on the fact that the temperature of fingertips or toes decreases significantly with smoking (159). However, deep inspiration in itself also causes vasoconstriction (p. 87). While it was thought that inhaling of tobacco smoke causes more vasoconstriction than inhaling of air, this does not seem to be quite clearly established (211). Findings of Abramson *et al.* (6) indicate that possibly the arteriovenous anastomoses are primarily implicated, because in the forearm no decrease of circulation can be demonstrated under the influence of tobacco, as contrasted with hands and feet. Franke and Hertzman (114) noted that the vessels of the forehead, too, are relatively unresponsive to smoking.

2. Vasodilators

Administration of *nicotinic acid* causes flushing of the face and trunk and a feeling

of warmth, sometimes also paresthesias. The increase in skin temperature is most marked over the ears, face, and neck, less marked over the trunk, and least over the extremities (262). An increase in blood flow, without appreciable increase in skin surface temperature, can be demonstrated plethysmographically (4) in hand and forearm. The marked peripheral vasodilator effect of nicotinic acid is in contrast with its not having any effect on the circulation of the brain (192). The natural coenzyme which acts as a vitamin is nicotinamide, and this compound has no dilator effect on cutaneous vessels. The free nicotinic acid ion which is responsible for the vasodilatation probably does not figure as a natural metabolite. Furthermore, in order to elicit measurable vasodilatation, nicotinic acid has to be given in unphysiologically large doses. Therefore, it is concluded that the vasodilator action of nicotinic acid is pharmacological, but not physiological. The tendency to cutaneous vasodilatation in nicotinamide deficiency also speaks against the assumption that nicotinic acid is a natural vasodilator metabolite.¹¹

Nitrites lessen the tone of the arterial and arteriolar muscle cells by direct action. In the fully developed response, vasodilatation in arterioles, capillaries, and venules can be observed. As a result of this response, cardiac output increases so as to maintain blood pressure. The two factors—lessened vascular tone and increased cardiac output—lead to a considerable increase of peripheral blood flow (266, 124), demonstrable also by direct capillary-microscopic observation (57). There is an initial arteriolar constriction if the subject is in an upright position (29), but this is not detectable in the horizontal position (286).

Large doses of *ergot alkaloids* have a sympathicolytic effect. By paralyzing the peripheral vasoconstrictor mechanism, they

11. L. Illig experimented a great deal with local (percutaneous) application of several nicotinic acid esters. These compounds cause not only active hyperemia but in the majority of normal test subjects also typical urticarial triple response (see reference, n. 2 on p. 81).

act indirectly as vasodilators. For instance, ergotamine tartrate administered intravenously in doses of 2 mg/kg body weight in the cat abolishes the vasoconstrictor responses of the skin to epinephrine. However, pharmacologically, a more important effect of ergot alkaloids than the sympathicolytic action of high doses is their direct constricting action on smooth muscles of peripheral arterioles, with consequent decrease in blood flow (5) and fall in peripheral skin temperature. Following the subcutaneous injection of as little as 0.5 mg. ergotamine tartrate in man, a definite decrease in blood flow can be demonstrated in the hand (5). In addition to constriction, ergot preparations cause endothelial injury in small arteries and arterioles and thus may cause gangrene. Although thrombosis is a prominent feature in ergot gangrene, its incidence is unaltered by the administration of heparin and dicumarol (14).

Ergotamine tartrate was highly recommended for symptomatic treatment of itching inflammatory skin diseases (39). The re-

ported beneficial effects may be due to a decrease in peripheral blood flow, with subsequent cooling, anti-inflammatory, and antipruritic effects. Apparently, in the recommended dosage no untoward effects were observed. However, a relatively slight overdosage may be dangerous (294). Dihydro derivatives of ergot alkaloids have considerably less vasospastic effect than have the parent-substances (36).

Priscol is a synthetic sympathicolytic and therefore a vasodilator substance which is now widely used therapeutically in peripheral vascular diseases. Although it was found to be fairly effective in these diseases (134), Allen (13) found surprisingly feeble effects on the skin of many healthy limbs after its administration.

The sensation of warmth in man after the *intravenous injection of calcium salts* is due not to vasodilatation but to stimulation of heat receptors. Such injections do not change the skin temperature (147) and do not alter the peripheral circulation in hands and legs.

XI. REACTIVE HYPEREMIA

If the circulation to a limb is obstructed for some time and then the occlusion is released, the volume of the limb increases, the blood flow is accelerated beyond normal, and the skin assumes a bright-red color. This phenomenon is called "reactive hyperemia."

The longer the occlusion lasts, the more prolonged is the reactive hyperemia. After an arterial occlusion lasting 2 minutes, the flush lasts $1\frac{1}{2}$ minutes; after an obstruction of 15 minutes, the redness persists for 8 minutes (186). A similar reaction follows venous congestion. As a matter of fact, quite low external pressure suffices to elicit reactive hyperemia. A just perceptible reaction is observed if 30 mm. Hg external pressure is maintained for 10 minutes (186); this pressure is sufficient to close the minute vessels of the skin.

Reactive hyperemia is independent of nervous impulses; it occurs in limbs after

complete nerve degeneration. Evidence was presented that it is due to the liberation of a metabolite in the tissue spaces. This substance is supposed to be liberated under the influence of anoxemia and to cause active vasodilatation in the minute vessels, which, in turn, leads to increased blood flow. Vasodilatation occurs during the phase of obstruction, but it can manifest itself only after the obstruction has been released. What the nature of this substance or substances is, has not been determined. The theory of Lewis (186) that it is an H-substance has not been substantiated (246). Landowne and Thompson (179) were unable to influence reactive hyperemia by administration of more than adequate doses of antihistaminic drugs.

Lewis (186) pointed out that, physiologically, some parts of the skin are practically always exposed to external pressure which is high enough to hinder circulation

at least in the papillary capillaries. In the prone position large parts of the skin of the trunk, in sitting of the buttocks, and in standing of the soles are subjected to such pressure. It might be significant that when the position is changed, an increased blood flow will compensate for the deficit suffered a moment earlier.

Reactive hyperemia was regarded as a special case of the general phenomenon of formation of local vasodilator metabolites if the tissues are undernourished. A regulatory mechanism in the opposite direction was recognized in the occurrence of anemic zones around hyperemic spots and of anemia developing in the spots themselves when the hyperemia had subsided. This can be well observed around the axon-reflex flare and within it after its subsidence. The formation of the anemic halo and the anemic spots is independent of nervous impulses and is not due to arterial contraction. Lewis (186) stated that anemic zones are not present around those hyperemic spots in which the increased flow apparently is needed for nutrition of the tissues (for instance, in local vasodilatation in response to external stim-

uli) but only in those in which the quantity of the blood is beyond the need of the tissues (for instance, in red flares) (186). Accordingly, Lewis finds that there are two kinds of small cutaneous telangiectases: those through which the blood flow is enhanced and which, therefore, have a pale halo and those in which there is no increased blood flow and which, therefore, have no halo (186).

Thus, in the presentation of Lewis (186), it appears that in the skin there is a balancing mechanism of the blood supply by locally formed metabolites. Metabolites are formed (or liberated) which open the minute vessels wherever there is lack of tissue nutrition; metabolites with the opposite effect appear and close them when there is excessive blood flow in the tissues.

Di Palma *et al.* (86) worked out a "ring test" which makes it possible to study reactive hyperemia on any small area of the skin surface. Milberg and Di Palma (205) studied the effect of pharmacological vehicles on this reaction. Some abnormalities of the reaction were found in lichen planus (205) and in the uninvolved skin in psoriasis (204).

XII. THE RELATION OF CIRCULATION IN THE SKIN AND IN INTERNAL ORGANS

In the skin proper, local vasomotor changes have a tendency to become generalized. This "law of consensual vascular reactions" (209) implies that vasomotor stimuli to circumscribed areas elicit the same kind of reaction (vasoconstriction or vasodilatation) all over the body surface. The discovery that such a tendency exists originated with Brown-Séquard (42). He found that the blood vessels of one hand contract if the other hand is immersed in cold water. The mechanism of such reactions was discussed on page 87. Also it was pointed out that the consensuality of the reaction is far from complete. For instance, more or less generalized vasoconstriction of trunk and extremities can be accompanied by vasodilatation of face, neck, and upper chest (p. 75).

Another "law" concerns the antagonistic behavior of peripheral and internal circulation. It was suggested by Dastré and Morat in 1884 (80) that vasodilatation in the peripheral circulation was always accompanied by vasoconstriction in the viscera, and similar antagonisms can be demonstrated in many other instances. There is little doubt that the redistribution of blood flow between internal organs and periphery is actually one way in which mammals adapt to changes of environmental temperatures (123). Various parts of the body may change their temperature within wide limits while the whole organism remains in a state of thermal balance (123) (chap. 10).

However, different internal organs seem to behave differently. Following heat stimuli to the skin, causing peripheral vasodilata-

tion, there is also a primary dilatation of the cerebral vessels (198). The kidneys react "consensually" with the skin to cutaneous temperature stimuli (198). The spleen contracts quite considerably in response to peripheral cold stimuli (267).

It was thought that a true antagonism existed between the circulation in the skin and that in the splanchnic area (210). But it must be doubted that this antagonism is always demonstrable, because the ingestion of substantial meals, connected with increased abdominal circulation, is associated with an increased rate of flow in the skin also (p. 245). Sjöstrand (258) found no antagonism at all between periphery and inner organs and emphasized that, on exercise when the blood flow in the muscles is greatly accelerated, there is increased blood flow in the skin also. He remarked, however, that, in general, the amount of blood circulating in the skin is relatively small as compared with other organs (p. 63). The most regularly demonstrable antagonism seems to exist between skin and lungs. Glaser (123) has shown that cooling results in movement of blood from the superficial vessels of the limbs to the lungs and that warming has the opposite effect.

The effect of cold and warm baths on the action of the heart seems to occur quite regularly. As Asher (15) stated first, cold acts like vagus nerve stimulation and heat like stimulation of the sympathetic. It was actually shown that the slowing effect of cold on the heartbeat is mediated by the vagus nerve; the reflex is abolished by vagotomy (198). The effects of cold and warm baths on blood pressure are not quite uniform (198).

Shifts in circulation following ultraviolet radiation of the whole body surface have frequently been discussed. Empirically, it was concluded that, at least under pathological conditions, there must be a change in the distribution of blood in internal organs, because after intense sunbathing "focal reactions," with greatly increased blood flow, often occur in tuberculous lung, bone, joint, and peritoneal and lymph-node lesions. In

the case of pulmonary tuberculosis the focal reactions frequently lead to profuse hemorrhages. That focal reactions actually occur and are connected with increased blood flow to the lesions can be directly seen if the lesions of patients with lupus vulgaris are covered and the rest of the body is intensely irradiated with sunlight: after such treatment the lesions become markedly hyperemic. This hyperemia seems to have some therapeutic action also. Jesionek showed that lupus vulgaris lesions may clear clinically by total body irradiations, in spite of tight covering of the lesions (158). Of course, this could be an effect also of vitamin D formation in the skin, independent of circulatory mechanisms.

In the past, physicians practicing heliotherapy of surgical tuberculosis often observed rapid cure of deep-seated lesions, for instance, in cases of tuberculosis of the hip joint (33), and such cures were attributed to continuous increased blood flow to the lesions elicited by sunbathing. Such an effect, if it exists, must be an indirect one, because the effective ultraviolet rays are completely absorbed in the superficial layers of the skin.

While the observations of therapeutic effects inside the body were not well controlled, it is a fact that general body irradiations with ultraviolet light cause a fall in systolic and diastolic blood pressure (139), long before the sunburn reaction develops. In my early studies I (241) showed that the fall in blood pressure can also be observed after mild irradiations with doses of ultraviolet rays which subsequently do not cause any reddening of the skin. The fall in blood pressure sets in right after the irradiation, progresses gradually, and the blood pressure reaches its lowest level on the next day. Low values may persist for several days. The phenomenon indicated decreased vascular tone in some internal organs which was not compensated by cardiac action. The early onset suggested that this effect was based on a nervous mechanism, and I pointed out that the state of the human body after generalized body irradiation with ultraviolet rays resembles the state of "sym-

pathetic hypotonus" partially reminiscent of what is seen in adrenal insufficiency: low blood pressure, low fasting blood sugar, flat glucose tolerance curves (242), and increased readiness to pigmentation (241). It seemed justifiable to assume that ultraviolet rays stimulate some afferent fibers in the skin directly or by chemical mediation and that the nerve impulses elicit vasodilatation in internal organs by some reflex mechanism.

This assumption was proved to be correct by the animal experiments of Von Reis and Sjöstrand (227). These authors measured the blood content of the liver and of the cortical part of the kidneys in guinea pigs and found it greatly increased in both sites after ultraviolet irradiation. Similar results were obtained after stimulation of the skin with mustard oil. When the cutaneous stimulation was done in denervated areas, the increase in blood content of liver and kidneys failed to appear. Thus this reaction seemed to be based exclusively on nerve impulses. Interestingly, the blood content of the lungs showed a decrease after cutaneous stimulation, a finding which fits well the more recent observations of Glaser (123) on the antagonistic behavior of cutaneous and pulmonary circulation, as contrasted with consensual reactions in other organs.

In addition to the nervous reflex phenomenon, the liberation of vasodilator substances in the skin by ultraviolet radiation may also contribute to the fall in blood pressure. In experiments on dogs, Von Kolnitz (166) found an increase in histamine content of the blood following exposure to ultraviolet light. This rise in histamine content paralleled the fall in blood pressure fairly closely when blood from the jugular

vein was analyzed. The correlation, however, was poor when blood from the cutaneous saphenous vein was used (167).

Following ingestion of substantial meals—apparently synergistically with vasodilatation in the splanchnic area—there is vasodilatation in the skin, demonstrable by a rise in skin surface temperature (52, 143, 37) and by the plethysmographic method (2). The cutaneous vasodilatation has a latent period of about $\frac{1}{2}$ hour and lasts for about 2 hours. Several substantial meals in succession will greatly prolong the duration of vasodilatation (239). The effect of meals containing mainly proteins is more pronounced than that of carbohydrate meals.

It is improbable that the mechanism of this reaction is a nervous one because of its long latent period. Koskowski (168) found that 1–3 hours after ingestion of a protein meal the blood contained vasodilator substances related to "peptones, tyramine and histamine." The circulation of such substances may well be the cause of peripheral vasodilatation. This interpretation is, at least, in harmony with the latent period and the greater effectiveness of protein meals. Whatever the mechanism of cutaneous vasodilatation after heavy meals may be, it is obvious that this reaction is of great clinical significance in the management of inflammatory skin diseases. Empirically, it has been known that rather sudden filling of the stomach is often followed by exacerbation of pruritus and inflammatory signs and that patients with inflammatory dermatoses get along better with small meals taken rather frequently than with single heavy meals.

The effect of alcohol intake on cutaneous circulation is discussed on pages 254–56.

BIBLIOGRAPHY

1. ABRAMSON, D. I. Vascular responses of man in health and disease. Chicago: University of Chicago Press, 1944.
2. ABRAMSON, D. I., and FIERST, S. M. Peripheral vascular response in man during digestion, *Am. J. Physiol.*, **133**:686–93, 1941.
3. ABRAMSON, D. I.; FIERST, S. M.; and FLOCHS, K. Evaluation of the local vasodilator effect of acetyl-beta methylcholine chloride (mecholy) by iontophoresis, *Am. Heart J.*, **23**:817–22, 1942.
4. ABRAMSON, D. I.; KATZENSTEIN, K. H.; and SENIOR, F. A. Effect of nicotinic acid on peripheral blood flow in man, *Am. J. M. Sc.*, **200**:96–102, 1940.

5. ABRAMSON, D. I., and LICHTMAN, S. S. Influence of ergotamine tartrate upon peripheral blood flow in subjects with liver disease, *Proc. Soc. Exper. Biol. & Med.*, **37**:262-67, 1937.
6. ABRAMSON, D. I.; ZAZEELA, H.; and OPPENHEIMER, B. S. Plethysmographic studies of peripheral blood flow in man. III. Effect of smoking upon the vascular beds in the hands, forearm and foot, *Am. Heart J.*, **18**:290-302, 1939.
7. ABRAMSON, D. I.; ZAZEELA, H.; and SCHKLOVEN, N. The vasodilating action of various therapeutic procedures which are used in the treatment of peripheral vascular disease; plethysmographic study, *Am. Heart J.*, **21**:756-66, 1941.
8. ABRAMSON, H. A. Whealing response of human skin to ultraviolet light and the histamine theory of allergic reactions, *Proc. Soc. Exper. Biol. & Med.*, **43**:410-12, 1940.
9. ADRIAN, E. D.; BRONK, D. W.; and PHILLIPS, G. Discharges in mammalian sympathetic nerves, *J. Physiol.*, **74**:115-33, 1932.
10. AHERN, J. J.; BARCLAY, W. R.; and EBERT, R. H. Modification of the rabbit ear chamber technic, *Science*, **110**:665, 1949.
11. AHLQUIST, R. P. Study of adrenergic receptors, *Am. J. Physiol.*, **153**:586-600, 1948.
12. ALDAO, C. N. G. Cutaneous forms of caisson disease, *Rev. argent. dermatosif.*, **33**:20-25, 1949.
13. ALLEN, W. J. The use of Priscol (2-benzyl-imidazoline-hydrochloride) as a vasodilator in man, *J. Physiol.*, **110**:27P, 1950.
14. ANDERSON, L. H., and WELLS, J. A. Attempts to prevent ergot gangrene with heparin and dicumarol. Vascular effects of ergot by fluorescein technique, *Proc. Soc. Exper. Biol. & Med.*, **67**:53-56, 1948.
15. ASHER, L. Beiträge zur Physiologie der Herznerven, *Verhandl. d. Kong. f. inn. Med.*, **21**:298-313, 1904.
16. BARCROFT, H., and HAMILTON, G. T. C. On the return of sudomotor and vasomotor reflexes to the sympathectomized hand, *J. Physiol.*, **108**:18P, 1949.
17. BARDY, H. Über Hemmung inflammatorischer Symptome, *Skandinav. Arch. f. Physiol.*, **32**:198-216, 1915.
18. BARGER, G., and DALE, H. H. Chemical structure and sympathomimetic action of amines, *J. Physiol.*, **41**:19-59, 1910-11.
19. BARKER, N. W.; HINES, E. A.; and CRAIG, W. McK. Livedo reticularis: a peripheral arteriolar disease, *Am. Heart J.*, **21**:592-604, 1941.
20. BARRON, D. H., and MATTHEWS, B. H. C. Dorsal root fibers, *J. Physiol.*, **83**:5P-6P, 1935.
21. ———. Recurrent fibers of the dorsal roots, *ibid.*, **85**:104-8, 1935.
22. BARSOUM, G. S., and GADDUM, J. H. Effect of cutaneous burns on blood-histamine, *Clin. Sc.*, **2**:357-62, 1936.
23. BARTON, D. S. A study of temperature and electric potentials in the menstrual cycle, *Yale J. Biol. & Med.*, **12**:503-23, 1940.
24. BAYLISS, W. M. On the origin from the spinal cord of the vasodilator fibers of the hind limb, and on the nature of these fibers, *J. Physiol.*, **26**:173-209, 1900-1901.
25. ———. Quoted in: BEST and TAYLOR, *The physiological basis of medical practice*. Baltimore: Williams & Wilkins, 1950.
26. ———. *The vasomotor system*. London and New York: Longmans, Green & Co., 1923.
27. BEAL, P. Studies in solar urticaria, *J. Invest. Dermat.*, **11**:415-33, 1948.
28. BEAN, W. B. A note on the development of cutaneous arterial spiders and palmar erythema in persons with liver disease and their development following the administration of estrogen, *Am. J. M. Sc.*, **204**:251-53, 1942.
29. BECK, W. C., and TAKATS, G. DE. The use of sodium nitrate for testing the flexibility of the peripheral vascular bed, *Am. Heart J.*, **15**:158-64, 1938.
30. BEECHER, H. K. Independent control of capillary circulation in mammals, *Skandinav. Arch. f. Physiol.*, **73**:1-6, 1936.
31. ———. Active control of all parts of capillary wall by sympathetic nervous system, *ibid.*, pp. 123-32.
32. BENEDICT, F. G., and PARMENTER, H. S. Human skin temperature as affected by muscular activity, exposure to cold, and wind movement, *Am. J. Physiol.*, **87**:633-53, 1928.
33. BERNHARD, O. *Sonnenlichtbehandlung in der Chirurgie*. Stuttgart: F. Enke, 1917.
34. BLUM, H. F.; BAER, R. L.; and SULZBERGER, M. B. Studies in hypersensitivity to light; urticaria solare ($\lambda < 3700 \text{ \AA}$), *J. Invest. Dermat.*, **7**:99-107, 1946.
35. BLUM, H. F.; BARKSDALE, E. E.; and GREEN, H. G. Urticaria solare (2400-

- 5000 Å), J. Invest. Dermat., **7**:109-15, 1946.
36. BLUNTSCHLI, H. J., and GOETZ, R. H. The effect of ergot derivatives on the circulation in man with special reference to two new hydrogenated compounds (dihydroergotamine and dehydroergocornine), Am. Heart J., **35**:873-94, 1948.
 37. BOOTH, G., and STRANG, J. M. Changes in temperature of the skin following the ingestion of food, Arch. Int. Med., **57**:533-43, 1936.
 38. BORDLEY, J.; GROW, M. H.; and SHERMAN, W. B. Intermittent blood flow in capillaries of human skin, Bull. Johns Hopkins Hosp., **62**:1-23, 1938.
 39. BRACK, W. Die Bedeutung des vegetativen Systems für die Entstehung des Juckens, Delib. Ninth Internat. Dermat. Cong., **1**:129-67. Budapest: Inst. Typogr. "Patria," 1935.
 40. BRESLAUER, F. Die Pathogenese der trophischen Gewebsschäden nach der Nervenverletzung, Deutsche Ztschr. f. Chir., **150**:50-81, 1919.
 41. BREWER, J. J. Rhythmic changes in the skin capillaries and their relation to menstruation, Am. J. Obst. & Gynec., **36**:597-612, 1938.
 42. BROWN-SÉQUARD. Quoted by RICHARDS (234).
 43. BRUCE, A. N. Über die Beziehung der sensiblen Nervenendigungen zum Entzündungsvorgang, Arch. f. exper. Path. u. Pharmacol., **63**:424-33, 1910.
 44. BÜLBRING, E., and BURN, J. H. Release of noradrenaline from adrenal medulla, J. Physiol., **109**:11P, 1949.
 45. ———. Liberation of noradrenaline from adrenal medulla by splanchnic stimulation, Nature, **163**:363, 1949.
 46. BURN, J. H. Action of tyramine and ephedrine, J. Pharmacol. & Exper. Therap., **46**:75-95, 1932.
 47. ———. Sympathetic vasodilator fibers, Physiol. Rev., **18**:137-53, 1938.
 48. ———. Relation of motor and inhibitor effects of local hormones, *ibid.*, **30**:177-93, 1950.
 49. BURTON, A. C. The range and variability of the blood flow in human fingers and the vasomotor regulation of body temperature, Am. J. Physiol., **127**:437-53, 1939.
 50. ———. Temperature regulation, Ann. Rev. Physiol., **1**:109-30, 1939.
 51. ———. The blood flow, temperature and color of the skin. In: Blood, heart and circulation, ed. MOULTON, F. R., pp. 308-13. ("A.A.A.S. Publications," No. 13.) Lancaster, Pa.: Science Press, 1940.
 52. BURTON, A. C., and MURLIN, J. R. Human calorimetry. III. Temperature distribution, blood flow and heat storage in body in basal condition and after ingestion of food, J. Nutrition, **9**:281-300, 1935.
 53. BURTON, A. C., and TAYLOR, R. M. Rhythmic fluctuations of sympathetic tone and their modification by temperature and by psychic influences, Am. J. Physiol., **126**:P453-54, 1939.
 54. ———. A study of the adjustment of peripheral vascular tone to the requirements of the regulation of body temperature, *ibid.*, **129**:565-77, 1940.
 55. CANNON, W. B., and ROSENBLUETH, A. Autonomic neuro-effector systems. New York: Macmillan Co., 1937.
 56. CARMICHAEL, E. A., and FRASER, F. R. The effects of acetylcholine in man, Heart, **16**:263-74, 1933.
 57. CARRIER, E. B. Studies on the physiology of capillaries. V. The reaction of the human skin capillaries to drugs and other stimuli, Am. J. Physiol., **61**:528-47, 1922.
 58. CARRIER, E. B., and REHBERG, P. B. Capillary and venous pressure in man, Skandinav. Arch. f. Physiol., **44**:20-31, 1923.
 59. CHAMBERS, R., and ZWEIFACH, B. W. Topography and function of the mesenteric capillary circulation, Am. J. Anat., **75**:173-205, 1944.
 60. ———. Intercellular cement and capillary permeability, Physiol. Rev., **27**:436-63, 1947.
 61. CLARK, E. R. Arterio-venous anastomoses, Physiol. Rev., **18**:229-47, 1938.
 62. CLARK, E. R., and CLARK, E. L. The relation of "Rouget" cells to capillary contractility, Am. J. Anat., **35**:265-82, 1925-26.
 63. ———. Observations on living preformed blood vessels as seen in a transparent chamber inserted into the rabbit's ear. b. Arteriovenous anastomoses, Anat. Rec., **45**:211, 1930.
 64. ———. The new formation of arteriovenous anastomoses in the rabbit's ear, Am. J. Anat., **55**:407-67, 1934.
 65. ———. Observations on changes in blood vascular endothelium in the living animal, *ibid.*, **57**:385-438, 1935.

66. ———. Caliber changes in minute blood vessels observed in the living animal, *ibid.*, **73**:215–50, 1943.
67. CLARK, E. R.; HITSCHLER, W. J.; KIRBY-SMITH, H. T.; REX, R. O.; and SMITH, J. H. General observations on the ingrowth of new blood vessels into standardized chambers in the rabbit's ear and the subsequent changes in the newly grown vessels over a period of months, *Anat. Rec.*, **50**:129–67, 1931.
68. CODE, C. F. Quantitative estimation of histamine in blood, *J. Physiol.*, **89**:257–68, 1937.
69. CODE, C. F., and MACDONALD, A. D. Histamine-like activity of blood, *Lancet*, **2**:730–33, 1937.
70. COHEN, T., and BENSON, S. Iontophoresis of acetyl- β -methylcholine chloride in peripheral vascular diseases, *Arch. Phys. Therapy*, **18**:583–87, 1937.
71. COON, J. M., and ROTHMAN, S. The nature of the pilomotor response to acetylcholine; some observations on the pharmacodynamics of the skin, *J. Pharmacol. & Exper. Therap.*, **68**:301–11, 1940.
72. COTTON, T. F.; SLADE, J. G.; and LEWIS, T. Observations upon dermatographism with special reference to the contractile power of capillaries, *Heart*, **6**:227–47, 1915–17.
73. COWIE, D. B.; FLEXNER, L. B.; and WILDE, D. S. Capillary permeability rate of transcapillary exchange of chloride in the guinea pig as determined with radiochloride, *Am. J. Physiol.*, **158**:231–36, 1949.
74. DALE, H. H. On some physiological actions of ergot, *J. Physiol.*, **34**:163–206, 1906.
75. ———. Chemical transmission of effects of nerve impulses (Linacre lecture), *Brit. M. J.*, **1**:835–41, 1934.
76. ———. Pharmacology and nerve endings, *Proc. Roy. Soc. Med.*, **28**:319–32, 1935.
77. DALE, H. H., and GADDUM, J. H. Reactions of denervated voluntary muscle and their bearing on mode of action of parasympathetic and related nerves, *J. Physiol.*, **70**:109–44, 1930.
78. DALE, H. H., and LAIDLAW, P. P. The physiological action of β -imidazolyl-ethylamine, *J. Physiol.*, **41**:318–44, 1910–11.
79. DALE, H. H.; LAIDLAW, P. P.; and RICHARDS, A. N. The action of histamine: its bearing on traumatic toxæmia as a factor in shock, *M. Res. Council Rept.* No. 26, pp. 8–15, 1919.
80. DASTRÉ and MORAT. Quoted by GLASER (123).
81. DEKANSKI, J. The effect of cutaneous burns on histamine in mice, *J. Physiol.*, **104**:151–60, 1945.
82. ———. The effect of protein hydrolysates (leukotaxine) on skin-histamine in cats, *ibid.*, **108**:233–45, 1949.
83. DE LALLA, V., JR. Causes in skin cooling in pressure breathing, deep inspiration and expiration, *Am. J. Physiol.*, **152**:122–30, 1948.
84. DILLON, J. B., and HERTZMAN, A. B. Vascular reactions in the nasal septum and skin of the head and digits, *Am. J. Physiol.*, **126**:P477–78, 1939.
85. DI PALMA, J. R.; REYNOLDS, S. R. M.; and FOSTER, F. I. Measurement of the sensitivity of the smallest blood vessels in human skin: responses to graded mechanical stimulation in normal men, *J. Clin. Investigation*, **20**:333–43, 1941.
86. ———. Quantitative measurement of reactive hyperemia in the human skin; individual and seasonal variations, *Am. Heart J.*, **23**:377–89, 1942.
87. DOUPE, J. Studies in denervation. B. The circulation in denervated digits, *J. Neurol. & Psychiat.*, N.S., **6**:97–111, 1943.
88. DOWNMAN, C. B. B.; GOGGIO, A. F.; MCSWINEY, B. A.; and YOUNG, M. H. C. Vasomotor responses from the paws of a cat, *J. Physiol.*, **96**:14P–15P, 1939.
89. DRAGSTEDT, C. A. Anaphylaxis, *Physiol. Rev.*, **21**:563–87, 1941.
90. ———. The significance of histamine in anaphylaxis, *J. Allergy*, **16**:69–77, 1945.
91. DRAGSTEDT, C. A.; WELLS, J. A.; and ROCHA E SILVA, M. Inhibitory effect of heparin and histamine released by trypsin, antigen and protease, *Proc. Soc. Exper. Biol. & Med.*, **51**:191–92, 1942.
92. EBBECKE, U. Die lokale vasomotorische Reaktion (L.V.R.) der Haut und inneren Organe, *Arch. f. d. ges. Physiol.*, **169**:1–81, 1917.
93. EBERT, R. H., and BARCLAY, W. R. Changes in connective tissue reaction induced by cortisone, *Ann. Int. Med.*, **37**:506–18, 1952.
94. EDWARDS, E. A.; HAMILTON, J. B.; and DUNTLEY, S. Q. Testosterone propionate as therapeutic agent in patients with organic disease of peripheral vessels; pre-

- liminary report, New England J. Med., **220**:865, 1939.
95. EICHNA, L. W., and BORDLEY, J. Capillary blood pressure in man: comparison of direct and indirect methods of measurement, J. Clin. Investigation, **18**:695-704, 1939.
 96. ELLINGER, F. Absorptionspektrum von Histidin und Histamin im Ultraviolett, Biochem. Ztschr., **215**:279-85, 1929.
 97. ———. Über die Entstehung eines Körpers mit histaminähnlichen Wirkungen aus Histidin unter Ultraviolettbestrahlung und die Bedeutung dieses Vorganges für das Lichterythem, Strahlentherapie, **38**:521-42, 1930.
 98. ELLIOT, A. H.; EVANS, R. D.; and STONE, C. S. Acrocyanosis: a study of the circulatory fault, Am. Heart J., **11**:431-43, 1936.
 99. ELLIS, L. B., and WEISS, S. Measurement of capillary pressure under natural conditions and after arteriolar dilatation in normal subjects and in patients with arterial hypertension and with arteriosclerosis, J. Clin. Investigation, **8**:47-67, 1929.
 100. ———. Study of cardiovascular responses in man to intravenous and intra-arterial injections of acetylcholine, J. Pharmacol. & Exper. Therap., **44**:235-51, 1932.
 101. EMMELIN, N. On the presence of histamine in plasma in physiologically active form, Acta physiol. Scandinav. (suppl. 34), **11**:1-71, 1945.
 102. EMMELIN, N., and FELDBERG, W. Pharmacologically active substances in the fluid of nettle hairs (*Urtica urens*), J. Physiol., **106**:14P-15P, 1947.
 103. EULER, U. S. VON. Sympathin in adrenergic nerve fibers, J. Physiol., **105**:26P, 1946.
 104. ———. Specific sympathomimetic ergone in adrenergic nerve fibres (sympathin) and its relation to adrenaline and nor-adrenaline, Acta physiol. Scandinav., **12**:73-97, 1946.
 105. ———. Sympathin, histamine, and acetylcholine in mammalian nerve, J. Physiol., **107**:10P-11P, 1948.
 106. EVANS, C. L. Dynamics of the circulation. In: STARLING, Principles of human physiology, pp. 609-41. 10th ed. Philadelphia: Lea & Febiger, 1949.
 107. EYSTER, W. H., JR.; ROTH, G. M.; and KIERLAND, R. R. Studies on the peripheral vascular physiology of patients with atopic dermatitis, J. Invest. Dermat., **18**:37-45, 1952.
 108. FARMER, L. Evaluation of the histamine intradermal test as a general indicator of allergy, J. Allergy, **16**:44-47, 1945.
 109. FEINBERG, S. M. Histamine and antihistaminic agents; their experimental and therapeutic status, J.A.M.A., **132**:702-13, 1946.
 110. FELDBERG, W. Histamine and anaphylaxis, Ann. Rev. Physiol., **3**:671-94, 1941.
 111. FERRIS, E. B., and ABRAMSON, D. I. Blood flow in the extremities under normal and abnormal conditions. In: Blood, heart and circulation, pp. 314-23. ("A.A.A.S. Publications," No. 13.) Lancaster, Pa.: Science Press, 1940.
 112. FLEISCH, A. Die Wirkung von Histamin, Acetylcholine und Adrenalin auf die Venen, Arch. f. d. ges. Physiol., **228**:351-72, 1931.
 113. FOERSTER, O. The dermatomes in man, Brain, **56**:1-39, 1933.
 114. FRANKE, F. E., and HERTZMAN, A. B. Effects of cigarette smoking on the skin circulation, Am. J. Physiol., **129**:P357, 1940.
 115. FREED, S. C., and LINDNER, E. The effect of steroids of the adrenal cortex and ovary on capillary permeability, Am. J. Physiol., **134**:258-62, 1941.
 116. FREEMAN, N. E. The effect of temperature on the rate of blood flow in the normal and in the sympathectomized hand, Am. J. Physiol., **113**:384-98, 1935.
 117. FREEMAN, N. E.; SMITHWICK, R. H.; and WHITE, J. C. Adrenal secretion in man. The reactions of the blood vessels of the human extremity, sensitized by sympathectomy to adrenalin and to adrenal secretion resulting from insulin hypoglycemia, Am. J. Physiol., **107**:529-34, 1934.
 118. GATÉ, J.; PELLERAT, J.; BADEL, M.; and MURAT, M. Recherches sur l'histaminémie. L'histaminurie en dermatologie et la taux physiologique de l'histamine cutanée, Compt. rend. Soc. de biol., **139**:542-44, 1944.
 119. GILLIAT, R. W.; GUTTMAN, L.; and WHITERIDGE, D. Inspiratory vasoconstriction in patients after spinal injuries, J. Physiol., **107**:67-75, 1948.
 120. GILJE, O. Capillary microscopy in the differential diagnosis of skin diseases, Acta dermat.-venereol., **33**:303-17, 1953.

121. ———. Capillary microscopic examination in skin diseases, *J. Invest. Dermat.* (to be published).
122. GILJE, O.; O'LEARY, P. A.; and BALDES, E. J. Capillary microscopic examination in skin diseases, *Arch. Dermat. & Syph.*, **68**:136-47, 1953.
123. GLASER, E. M. The effects of cooling and warming on the vital capacity, forearm and hand volume, and skin temperature of man, *J. Physiol.*, **109**:421-29, 1949.
124. GÖPFERT, H.; GROSS, F.; and MATTHES, K. Über die Wirkung gefässaktiver Substanzen auf den peripheren Kreislauf beim Menschen, *Arch. f. exper. Path. u. Pharmacol.*, **195**:93-116, 1940.
125. GOETZ, R. H. Nervous control of blood-flow through skin as studied by effect of adrenaline, *Quart. J. Exper. Physiol.*, **29**:239-57, 1939.
126. GRANROTH, T., and NILZEN, A. On the histaminolytic activity of skin extracts, *Acta physiol. Scandinav.*, **15**:188-92, 1948.
127. GRANT, R. T., and BLAND, E. F. Observation on arteriovenous anastomoses in human skin and in bird's foot with special reference to the reaction to cold, *Heart*, **15**:385-407, 1931.
128. ———. Observations on the vessels and nerves of the rabbit's ears with special reference to cold, *ibid.*, **16**:69-101, 1932.
129. GRANT, R. T., and HOLLING, H. E. Further observation on the vascular responses of the human limb to body warming; evidence for sympathetic vasodilator nerves in the normal subject, *Clin. Sc.*, **3**:273-85, 1938.
130. GRANT, R. T., and PEARSON, R. S. B. The blood circulation in the human limb: observations on the differences between the proximal and distal parts and remarks on the regulation of body temperature, *Clin. Sc.*, **3**:119-39, 1938.
131. GRANT, R. T.; PEARSON, R. S. B.; and COMEAU, W. J. Observations on urticaria provoked by emotion, by exercise and by warming the body, *Clin. Sc.*, **2**:253-72, 1936.
132. GRAYBIEL, A., and GLENDY, R. E. Circulatory effects following the intravenous administration of pitressin in normal persons and in patients with hypertension and angina pectoris, *Am. Heart J.*, **21**:481-89, 1941.
133. GRIMSON, K. S. Sympathectomy and the circulation. Anatomic and physiologic considerations and early and late limitations, *Surgery*, **19**:277-98, 1946.
134. GRIMSON, K. S.; REARDON, M. J.; MARZONI, F. A.; and HENDRIX, J. P. The effects of Priscol (2-benzyl-2-imidazoline hydrochloride) on peripheral vascular diseases, hypertension and circulation in patients, *Ann. Surg.*, **127**:968-90, 1948.
135. GROLLMAN, A., and GEILING, E. M. K. The cardiovascular and metabolic reactions of man to intramuscular injections of posterior pituitary liquid (pituiratin, pitressin and pitocin), *J. Pharmacol. & Exper. Therap.*, **46**:447-60, 1932.
136. HAGEN, W. Periodische, konstitutionelle und pathologische Schwankungen im Verhalten der Blutcapillaren, *Arch. f. path. Anat.*, **239**:504-56, 1922.
137. HARMER, I. M., and HARRIS, K. E. Vascular reactions in man in response to histamine, *Heart*, **13**:381-94, 1926.
138. HARVEY, A. M.; LILIENHAL, J. R., JR.; and TALBOT, S. A. On the effects of intra-arterial injection of acetylcholine and Prostigmine in normal man, *Bull. Johns Hopkins Hosp.*, **69**:529-46, 1941.
139. HASSELBALCH, K. A. Die Wirkungen des chemischen Lichtbades auf Respiration und Blutdruck, *Skandinav. Arch. f. Physiol.*, **17**:431-72, 1905.
140. HECHTOR, O.; KROHN, L.; and HARRIS, J. Effects of estrogens and other steroids in capillary permeability, *Endocrinology*, **30**:598-608, 1942.
141. HEIMBERGER, H. Beiträge zur Physiologie der menschlichen Capillaren. III. Verhalten auf Reizung mit galvanischem Strom, *Ztschr. f. d. ges. exper. Med.*, **51**:112-23, 1926.
142. HELLAUER, H., and UMRATH, K. The transmitter substance of sensory nerve fibers, *J. Physiol.*, **106**:20P-21P, 1947.
143. HERRICK, J. F., and SHEARD, C. Changes in surface and rectal temperatures in man produced by the ingestion of food subsequent to twenty-four hour fast, *Am. J. Physiol.*, **113**:62, 1936.
144. HERTZMAN, A. B. Photoelectric plethysmography of fingers and toes in man, *Proc. Soc. Exper. Biol. & Med.*, **37**:529-34, 1937.
145. ———. The blood supply of various skin areas as estimated by the photoelectric plethysmograph, *Am. J. Physiol.*, **124**:328-40, 1938.
146. HEYMANS, C., and BAYLESS, F. Sur l'action

- circulatoire de la b \acute{e} ta-*p*-oxyph \acute{e} nyl-isopropyl-m \acute{e} thylamine, Arch. internat. de pharmacodyn. et de th \acute{e} rap., **56**:319-26, 1937.
147. HOFF, H. E.; SMITH, P. N.; and WINKLER, A. W. The relation of blood pressure and concentration in serum of potassium, calcium, and magnesium, Am. J. Physiol., **127**:722-30, 1939.
 148. HOLLING, H. E. Observations on the oxygen content of venous blood from the arm vein and on the oxygen consumption of resting human muscle, Clin. Sc., **4**:103-11, 1939.
 149. HOPKINS, J. G.; KESTEN, B. M.; and HAZEL, O. G. Urticaria provoked by heat or by psychic stimuli, Arch. Dermat. & Syph., **38**:679-91, 1938.
 150. HORTON, B. T.; BROWN, G. E.; and ROTH, G. M. Hypersensitiveness to cold; with local and systemic manifestations of a histamine-like character: its amenability to treatment, J.A.M.A., **107**:1263-69, 1936.
 151. HOSKINS, R. G.; GUNNING, R. E. L.; and BERRY, E. L. The effects on the distribution of the blood. I. Volume changes and venous discharge in the limbs, Am. J. Physiol., **41**:513-28, 1916.
 152. HUFF, S. E.; TAYLOR, H. L.; and KEYS, A. Observations on the peripheral blood flow in chronic lupus erythematosus, J. Invest. Dermat., **14**:21-36, 1950.
 153. HYNDMAN, O. R., and WOLKIN, J. The autonomic mechanism of heat conservation and dissipation. I. Effects of heating the body; evidence for the existence of capillary dilator nerves in anterior roots, Am. Heart J., **22**:289-304, 1941.
 154. INGRAM, P. W. Quoted by RICHARDS (234).
 155. JADASSOHN, J. Dermatologie. 2d ed. Vienna and Bern: Verlag f. Medizin, Weidman & Co., 1938.
 156. JANOWITZ, H., and GROSSMAN, M. I. Minimal effective dose of intravenously administered histamine in pregnant and non-pregnant human beings, Am. J. Physiol., **157**:94-98, 1949.
 157. JANOWITZ, H.; SONNENSCHN, R. R.; and GROSSMAN, M. I. Adrenotropic receptors of the human skin, J. Invest. Dermat., **12**:205-6, 1949.
 158. JESIONEK, A. Heliotherapie und Pigment, Ztschr. f. Tuberk., **24**:401-14, 1915.
 159. JOHNSON, H. J., and SHORT, J. J. The effect of smoking on skin temperature, J. Lab. & Clin. Med., **19**:962-66, 1934.
 160. KATZ, G. Histamine release from blood cells in anaphylaxis in vitro, Science, **91**:221, 1940.
 161. KEYS, A., and VIOLANTE, A. Cardiocirculatory effects in man of Neosynephrine (1- α -hydroxy- β -methylamino-3-hydroxyethylbenzene hydrochloride), J. Clin. Investigation, **21**:1-12, 1942.
 162. KIRK, E. Untersuchungen \ddot{u} ber den Einfluss des normalen Schlafes auf die Temperatur der F \ddot{u} sse, Skandinav. Arch. f. Physiol., **61**:71-78, 1931.
 163. KNISELY, M. H.; BLOCH, E. H.; ELIOT, T. S.; and WARNER, C. Sludged blood, Science, **106**:431-40, 1948.
 164. KOELLE, G. B., and GILMAN, A. Anticholinesterase drugs, J. Pharmacol. & Exper. Therap., **95**:166-216, 1949.
 165. KOHLER, R., and WETH, G. Die Wirkung der cervicalen Sympathektomie auf die Angina pectoris und die Ausfallserscheinungen nach diesem operativen Eingriff, Ztschr. f. klin. Med., **99**:205-31, 1924.
 166. KOLNITZ, H. VON. Blood and blood pressure changes following carbon arc irradiation. I. A micro method for the estimation of histamine in blood, Am. J. Physiol., **126**:P557-58, 1939.
 167. ———. Blood and blood pressure changes following carbon arc irradiation. II. The correlation between blood histamine and blood pressure, *ibid.*, **129**:P399-P400, 1940.
 168. KOSKOWSKI, W. Les propri \acute{e} t \acute{e} s dynamiques du sang au cours de la digestion et leurs changements dans diff \acute{e} rentes conditions exp \acute{e} rimentales, J. de physiol. et de path. g \acute{e} n., **31**:697-712, 1933.
 169. KOVACS, R., and KOVACS, J. Newer aspects of iontophoresis for arthritis and circulatory disturbances, Arch. Phys. Therapy, **15**:593-98, 1934.
 170. KROGH, A. The anatomy and physiology of capillaries. New Haven, Conn.: Yale University Press, 1929; third printing, 1936.
 171. KUNKEL, P.; STEAD, E. A., JR.; and WEISS, S. Blood flow and vasomotor reactions in the hand, forearm, foot, and calf in response to physical and chemical stimuli, J. Clin. Investigation, **18**:225-38, 1939.
 172. KWIATOWSKI, H., Histamine in nervous tissue, J. Physiol., **102**:32-41, 1943.

173. LAMBERT, E., and ROSENTHAL, S. R. Study of skin histamine (with some results of splanchnic nerve stimulation), *Proc. Soc. Exper. Biol. & Med.*, **52**:302-4, 1943.
174. LANDIS, E. M. The capillary pressure in frog mesentery as determined by micro-injection methods, *Am. J. Physiol.*, **75**:548-70, 1925-26.
175. ———. The capillary blood pressure in mammalian mesentery as determined by the micro-injection method, *ibid.*, **93**:353-62, 1930.
176. ———. Micro-injection studies of capillary blood pressure in human skin, *Heart*, **15**:209-28, 1930.
177. ———. Capillary pressure and capillary permeability, *Physiol. Rev.*, **14**:404-81, 1934.
178. ———. Peripheral circulation, *Ann. Rev. Physiol.*, **2**:125-50, 1940.
179. LANDOWNE, M., and THOMPSON, W. S. Failure of antihistaminic drugs to reduce reactive hyperemia in man, *Proc. Soc. Exper. Biol. & Med.*, **69**:537-42, 1948.
180. LANGLEY, J. N. Antidromic action. I, *J. Physiol.*, **57**:428-46, 1922-23.
181. ———. Antidromic action. II. Stimulation of the peripheral nerves of the cat's hind foot, *ibid.*, **58**:49-69, 1923-24.
182. LANGLEY, J. N., and ANDERSON, H. K. On reflex action from sympathetic ganglia, *J. Physiol.*, **16**:410-40, 1894.
183. LEHNER, E., and KENEDY, D. Weitere Beiträge zur Kenntnis der Entzündungen der Haut mit netzförmiger oder verästelter Zeichnung. *Inflammatio cutis racemosa*, *Arch. f. Dermat. u. Syph.*, **149**:387-92, 1925.
184. LENNARTZ, E. Die Reaktion der Capillaren auf mechanische Reize bei Nichtschwängern, Schwängern und Wöchnerinnen, *Arch. f. d. ges. Physiol.*, **191**:302-11, 1921.
185. LEVINE, R.; GOLDSTEIN, M. S.; RAMEY, E. R.; and FRITZ, I. Studies on the action of cortisone in stress situations, *Bull. New England M. Center*, **13**:114-20, 1951.
186. LEWIS, T. The blood vessels of the human skin and their responses. London: Shaw & Sons, 1927.
187. ———. Observations upon the vascular axon reflex in human skin, as exhibited by a case of urticaria, with remarks upon the nocifensor nerve hypothesis, *Clin. Sc.*, **4**:365-84, 1942.
188. LEWIS, T., and GRANT, R. T. Vascular reactions of the skin to injury. II. The liberation of a histamine-like substance in injured skin; the underlying cause of factitious urticaria and of wheals produced by burning; and observations upon the nervous control of certain skin reactions, *Heart*, **11**:209-65, 1924.
189. LEWIS, T., and MARVIN, H. M. Observations relating to vasodilatation arising from antidromic impulses, to herpes zoster and trophic effects, *Heart*, **14**:27-48, 1927.
190. LEWIS, T., and PICKERING, G. W. Vasodilatation in the limbs in response to warming the body, with evidence for sympathetic vasodilator nerves in man, *Heart*, **16**:33-51, 1931.
191. LOBITZ, W. C., JR., and CAMPBELL, C. J. Physiologic studies in atopic dermatitis; disseminated neurodermatitis. I. The local cutaneous response to intradermally injected acetylcholine and epinephrine, *Arch. Dermat. & Syph.*, **67**:575-89, 1953.
192. LOMAN, J.; RINKEL, M.; and MYERSON, A. The intracranial and peripheral vascular effects of nicotinic acid, *Am. J. M. Sc.*, **202**:211-16, 1941.
193. LORINCZ, A. L. Personal communication.
194. MACINTOSH, F. C., and PATON, W. D. M. The liberation of histamine by certain organic bases, *J. Physiol.*, **109**:190-219, 1949.
195. MALMÉJAC, J.; DONNET, V.; and JONESCO, G. Sur l'action vasomotrice cutanée de très faibles quantités d'adrénaline, *Compt. rend. Soc. de biol.*, **130**:560-62, 1939.
196. MARSH, D. F.; PELLETIER, M. H.; and ROSS, C. A. The comparative pharmacology of *N*-alkylarterenols, *J. Pharmacol. & Exper. Therap.*, **92**:108-20, 1948.
197. MASSON, P. Le glomus neuromyoartériel des régions tactiles et ses tumeurs. Les glomus neurovasculaires. *Histophysiologie of Policard*. Paris: Hermann & Co., 1937.
198. MATTHES. Quoted by SCHILF, E., *Reflexe von der Haut und den inneren Organen auf Herz und Gefässe*. In: BETHE, *Handb. d. norm. u. path. Physiol.*, **16**/2:1163-1206, Berlin: J. Springer, 1931.
199. MAYER, R. L. Pyribenzamine in experimental nonallergic and allergic dermatitis, *J. Invest. Dermat.*, **8**:67-79, 1947.
200. MEMMESHEIMER, A. M. Zur Pathogenese der sogenannten essentialen Teleangiectasien, *Dermat. Ztschr.*, **53**:399-413, 1928.
201. MENKIN, V. The role of inflammation in immunity, *Physiol. Rev.*, **18**:366-418, 1938.

202. MENKIN, V. Dynamics of inflammation. New York: Macmillan Co., 1940. See also: Modern views on inflammation, *Internat. Arch. Allergy*, **4**:131-68, 1953.
203. ———. Effect of adrenal cortex extract on capillary permeability, *Am. J. Physiol.*, **129**:691-97, 1940.
204. MILBERG, I. L. The reactive hyperemia response of the uninvolved skin of patients with psoriasis, *J. Invest. Dermat.*, **9**:31-39, 1947.
205. MILBERG, I. L., and DI PALMA, J. R. The effects of hydrophilic bases and salt solutions upon reactive hyperemia in the skin: application in a case of lichen ruber planus, *J. Invest. Dermat.*, **5**:403-10, 1942.
206. MOSS, J. N.; BEILER, J. M.; and MARTIN, G. J. Inhibitions of anaphylaxis in guinea pigs by *D*-catechin, *Science*, **112**:16, 1950.
207. MÜLLER, L. R. Studien über den Dermographismus und dessen diagnostische Bedeutung, *Deutsche Ztschr. f. Nervenheilk.*, **47-48**:413-35, 1913.
208. MÜLLER, O. Die feinsten Blutgefäße des Menschen in gesunden und kranken Tagen. Vol. **1**: Zur normalen Anatomie und Physiologie sowie allgemeinen Pathologie des feinsten Gefäßabschnittes beim Menschen. Stuttgart: F. Enke, 1937.
209. ———. *Ibid.*, Vol. **2**: Zur speziellen Pathologie des feinsten Gefäßabschnittes beim Menschen. Stuttgart: F. Enke, 1939.
210. MÜLLER, O., and VEIEL, E. Beiträge zur Kreislaufphysiologie des Menschen, besonders zur Lehre von der Blutverteilung. I, *Samml. klin. Vort.*, Nos. 606-8, pp. 641-724, 1910.
211. MULINOS, M. G., and SHULMAN, I. The effects of cigarette smoking and deep breathing on the peripheral vascular system, *Am. J. M. Sc.*, **199**:708-20, 1940.
212. NESTEROW. Quoted by MÜLLER, O., *Die Kapillaren der menschlichen Körperoberfläche in gesunden und kranken Tagen*. Stuttgart: F. Enke, 1922.
213. NICKERSON, M. The pharmacology of adrenergic blockade, *J. Pharmacol. & Exper. Therap.*, **95**:27-101, 1949.
214. NILZEN, A. Studies in histamine (H-substance) with special reference to conditions obtaining in urticaria and related skin-changes, *Acta dermat.-venereol. (suppl. 17)*, **27**:1-68, 1949.
215. NORRLIND, R. Prurigo Besnier (atopic dermatitis): clinical-experimental study of its pathogenesis with special reference to acute infections of the respiratory tract, *Acta dermat.-venereol. (suppl. 13)*, **26**:1-168, 1946.
216. O'LEARY, P. A.; MONTGOMERY, H.; and BRUNSTING, L. A. Livedo reticularis; recurring ulcerations of the ankles in the summer, *Arch. Dermat. & Syph.*, **50**:213, 1944.
217. PARRISIUS. Quoted by MÜLLER, O. (209).
218. PELLERAT, J., and MURAT, M. Variations de l'histamine cutanée et cuti-réactions à la tuberculine, *Compt. rend Soc. de biol.*, **138**:574-75, 1944.
219. ———. Variations de la teneur cutanée en histamine sous l'influence du froid et dans certaines dermatoses, *ibid.*, **139**:1141-42, 1945.
220. PERCIVAL, G. H., and SCOTT, C. M. Study of skin vessels in some forms of inflammation of skin, *J. Pharmacol. & Exper. Therap.*, **41**:147-63, 1931.
221. PERLA, D.; FREIMAN, D. G.; SANDBERG, M.; and GREENBERG, S. S. Prevention of histamine and surgical shock by cortical hormone (desoxycorticosterone acetate and cortin) and saline, *Proc. Soc. Exper. Biol. & Med.*, **43**:397-404, 1940.
222. PETERSEN, W. F., and MILLES, G. The relation of menstruation to the permeability of the skin capillaries and the autonomic tonus of the skin vessels, *Arch. Int. Med.*, **38**:730-35, 1926.
223. PETERSEN, W. F., and WILLIS, D. A. Capillary permeability and inflammatory index of the skin in the normal person as determined by blister, *Arch. Int. Med.*, **38**:663-81, 1926.
224. PISCHZEK, F., and SCHMIDT, P. Über Capillarbeobachtungen an Schwangeren, *Zentralbl. f. Gynäk.*, **50**:578-85, 1926.
225. RANSON, S. W., and BILLINGSLEY, P. R. Vasomotor reactions from stimulation of the floor of the fourth ventricle. Studies in vasomotor reflex arcs. III, *Am. J. Physiol.*, **41**:85-90, 1916.
226. REHBERG, P. B., and CARRIER, E. B. Studies on the physiology of capillaries. V. Concerning the reaction of the human skin capillaries to venous blood, *Skandinav. Arch. f. Physiol.*, **42**:250-65, 1922.
227. REIS, G. VON, and SJÖSTRAND, F. Über die Einwirkung von Hauteizungen mit Senföl und ultravioletter Bestrahlung auf die periphere Blutverteilung, *Skandinav. Arch. f. Physiol.*, **79**:139-55, 1938.

228. REYNOLDS, S. R. M. Dermovascular action of estrogen, the ovarian follicular hormone, *J. Invest. Dermat.*, **4**:7-22, 1941.
229. REYNOLDS, S. R. M., and FOSTER, F. I. Peripheral vascular action of estrogen in the human male, *J. Clin. Investigation*, **18**:649-55, 1939.
230. ———. Peripheral vascular action of estrogen observed in the ear of rabbit, *J. Pharmacol. & Exper. Therap.*, **68**:173-84, 1940.
231. REYNOLDS, S. R. M.; HAMILTON, J. B.; DI PALMA, J. R.; HUBERT, G. R.; and FOSTER, F. I. Dermovascular action of certain steroid hormones in castrate, eunuchoid and normal men, *J. Clin. Endocrinol.*, **2**:228-36, 1942.
232. REYNOLDS, S. R. M.; KAMINESTER, S.; FOSTER, F. I.; and SCHLOSS, S. Dermovascular effects of estrogen in women with menopausal flushes, *Surg., Gynec. & Obst.*, **73**:206-11, 1941.
233. REYNOLDS, S. R. M., and DI PALMA, J. R. Dermovascular changes during the menstrual cycle: failure to find a cyclic variation in contractile or dilating capacity of capillaries of the skin, *J. Clin. Endocrinol.*, **2**:226-27, 1942.
234. RICHARDS, R. L. The peripheral circulation in health and disease. Edinburgh: E. & S. Livingstone, Ltd., 1946.
235. ROCHA E SILVA, M.; SCROGGIE, A. E.; FIDLAR, E.; and JAKES, L. B. Liberation of histamine and heparin by peptone from the isolated dog's liver, *Proc. Soc. Exper. Biol. & Med.*, **64**:141-46, 1947.
236. ROGERS, M. P. Use of a sympatholytic drug (Priscol). From the point of view of a general surgical practitioner, *J.A.M.A.*, **140**:272-76, 1949.
237. ROOME, N. W. The effects of intra-arterial epinephrine on the blood flow in an extremity, *Am. J. Physiol.*, **123**:543-49, 1938.
238. ROSE, B., and BROWNE, J. S. L. Studies on blood histamine in cases of burns, *Ann. Surg.*, **115**:390-99, 1942.
239. ROTH, G. M., and SHEARD, C. Maintenance of vasodilation of the extremities of normal individuals for a prolonged period by the ingestion of two to four substantial meals in close succession, *Am. J. Physiol.*, **152**:183-88, 1948.
240. ROTHMAN, S. Unpublished experiments made in 1925.
241. ———. Untersuchungen über die Physiologie der Lichtwirkungen, *Ztschr. f. d. ges. exper. Med.*, **36**:398-446, 1923.
242. ———. Erhöhung der Zuckertoleranz durch Lichtbäder, *Klin. Wchnschr.*, **3**:1959, 1924.
243. ———. The role of the autonomic nervous system in cutaneous disorders, *Psychosom. Med.*, **7**:90-96, 1945.
244. ROTHMAN, S., and COON, J. M. Studies on liberation of acetylcholine in the skin, *J. Invest. Dermat.*, **3**:99-106, 1940.
245. ROUS, P., and GILDING, H. P. The meaning of Bier's spots, *Proc. Soc. Exper. Biol. & Med.*, **26**:497-98, 1929.
246. ———. Is the local vasodilation after different tissue injuries referable to a single cause? *J. Exper. Med.*, **51**:27-39, 1930.
247. ROUS, P.; GILDING, H. P.; and SMITH, F. Gradient of vascular permeability, *J. Exper. Med.*, **51**:807-30, 1930.
248. RUBIN, L.; BEAL, P. L.; and ROTHMAN, S. Method for protection of patients with solar urticaria, *J. Invest. Dermat.*, **8**:199, 1947.
249. SACKS, B. Observations upon the vascular reactions in man in response to infundin with special reference to the behavior of capillaries and venules, *Heart*, **11**:353-70, 1924.
250. SANDERS, A. G.; EBERT, R. H.; and FLOREY, H. W. The mechanism of capillary contraction, *Quart. J. Exper. Physiol.*, **30**:281-87, 1940.
251. SANDISON, J. C. Transparent chamber of rabbit's ear, giving complete description of improved technic of construction and introduction and general account of growth and behavior of living cells and tissues as seen with the microscope, *Am. J. Anat.*, **41**:447-73, 1928.
252. SARNOFF, J., and SIMEONE, F. A. Vasodilator fibers in the human skin, *J. Clin. Investigation*, **26**:453-59, 1947.
253. SCHAFER, E. P. S. Testosterone propionate and the vasomotor phenomena of gonadal deficiency, *Lancet*, **238**:161-64, 1940.
254. SCHNEIDER, D. Klinische Erfahrungen mit dem Kreislaufmittel "Veritol," *Klin. Wchnschr.*, **16**:736-40, 1937.
255. SCHORR, E. The participation of hepatorenal factors in experimental renal hypertension. In: BELL, E. T. (ed.), *Hypertension: a symposium held at the University of Minnesota, September 18-20, 1950*, pp. 79-93. Minneapolis: University of Minnesota Press, 1951.

256. SENEAR, F. E., and STAFF. Perniones and livedo reticularis in a poliomyelitic limb, *Arch. Dermat. & Syph.*, **60**:865, 1949.
257. SERRA, V. Sul significato degli angiomi rubino, *Osp. maggiore*, **20**:261-73, 1932.
258. SJÖSTRAND, T. On the distribution of the blood in the peripheral vascular system, *Skandinav. Arch. f. Physiol. (suppl.)*, **71**:1-150, 1935.
259. SOLLMAN, T. H. A manual of pharmacology. 7th ed. Philadelphia and New York: W. B. Saunders Co., 1948.
260. SPALTEHOLZ, W. Die Vertheilung der Blutgefäße in der Haut, *Arch. f. Anat. u. Physiol. (Anat. Abt.)*, pp. 1-54, 1893.
261. ———. Blutgefäße der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1/1**:379-433. Berlin: J. Springer, 1927.
262. SPIES, T. D.; BEAN, W. B.; and STONE, R. E. Treatment of subclinical and classic pellagra; use of nicotinic acid amide and sodium nicotinate, with special reference to the vasodilator action and the effect on mental symptoms, *J.A.M.A.*, **111**:584-92, 1938.
263. STARLING, E. H. On the absorption of fluids from the connective tissue spaces, *J. Physiol.*, **19**:312-26, 1895-96.
264. STEAD, E. A., JR.; KUNKEL, P.; and WEISS, S. Effect of pitressin in circulatory collapse induced by sodium nitrite, *J. Clin. Investigation*, **18**:673-78, 1939.
265. STEIN, I. D.; HARPUDER, K.; and BYER, J. Effect of sympathectomy in the human limb, *Am. J. Physiol.*, **152**:499-504, 1948.
266. STEWART, G. N. Studies on the circulation in man. XV. Further observations, chiefly pharmacological, on the criteria by which deficiencies in the blood flow (in the hands or feet) due to mechanical causes may be discriminated from changes due to functional (vasomotor) causes, *J. Pharmacol. & Exper. Therap.*, **7**:281-93, 1915.
267. STRASSER, A., and WOLF, H. Über die Blutversorgung der Milz, *Arch. f. d. ges. Physiol.*, **108**:590-626, 1905.
268. STRICKER, S. Untersuchungen über die Gefässnerven-Wurzeln des Ischiadicus, *Sitzungsb. königl. Akad. d. Wissensch., wiss. Math. Cl.*, **74**:Abt. III, 313-32, 1876.
269. SULZBERGER, M. B. Dermatologic allergy; an introduction in the form of a series of lectures. Springfield, Ill., and Baltimore: Charles C Thomas, 1940.
270. SULZBERGER, M. B., and BAER, R. L. Studies in hypersensitivity to light. I. Preliminary report, *J. Invest. Dermat.*, **6**:345-48, 1945.
271. TARRAS-WAHLBERG, B. Über den Histamingehalt der Haut nach Ultraviolettbestrahlung, *Klin. Wchnschr.*, **16**:958-60, 1937.
272. TÖRÖK, L. Urticaria. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **6/2**:145-215. Berlin: J. Springer, 1928.
273. TOSATTI (MOLINARI TOSATTI, P.). Transporte passivo alla Prausnitz e Küstner in un caso di urticaria factitia, *Policlinico (sez. med.)*, **43**:205-23, 1936.
274. TOWER, S. S. Pain; definition and properties of unit for sensory perception, *A. Res. Nerv. & Ment. Dis. Proc.*, **23**:16-43, 1943.
275. UMRATH, K., and HELLAUER, H. F. Das Vorkommen der sensiblen Substanz und von Aktionssubstanzen abbauenden Fermenten, *Arch. f. d. ges. Physiol.*, **250**:737-46, 1948.
276. VIMTRUP, B. Beiträge zur Anatomie der Capillaren. I. Über contractile Elemente in der Gefäßwand der Blutcapillaren, *Ztschr. f. Anat. u. Entwcklungsgesch.*, **55**:150-82, 1922.
277. ———. Beiträge zur Anatomie der Capillaren. II. Weitere Untersuchungen über contractile Elemente in der Gefäßwand der Blutcapillaren, *ibid.*, **68**:469-82, 1923.
278. WALSHE, F. M. R. The anatomy and physiology of cutaneous sensibility: a critical review, *Brain*, **65**:48-112, 1942.
279. WARREN, J. V.; WALTER, C. W.; ROMANO, J.; and STEAD, E. A., JR. Blood flow in the hand and forearm after paravertebral block of the sympathetic ganglia. Evidence against sympathetic vasodilator nerves in the extremities of man, *J. Clin. Investigation*, **21**:665-73, 1942.
280. WEISS, S.; ROBB, G. P.; and ELLIS, L. B. The systemic effects of histamine in man: with special reference to the responses of the cardiovascular system, *Arch. Int. Med.*, **49**:360-96, 1932.
281. WEST, G. B. The active principle of the adrenal medulla, *J. Physiol.*, **110**:9P-10P, 1950.
282. WETZEL, N. C., and ZOTTERMAN, Y. On differences in the vascular colouration of various regions of the normal human skin, *Heart*, **13**:358-70, 1926.
283. WHITCHER, C. E., and GRIFFITH, F. R., JR. A possible role of the skin in the effect

- of adrenalin on body temperature and respiratory metabolism, *Am. J. Physiol.*, **156**:114-16, 1949.
284. WHITFIELD, A. On the white reaction (white line) in dermatology, *Brit. J. Dermat.*, **50**:71-82, 1938.
 285. WILKINS, R. W.; DOUPE, J.; and NEWMAN, H. W. Rate of blood flow in normal fingers, *Clin. Sc.*, **3**:403-11, 1938.
 286. WILKINS, R. W.; HAYNES, F. W.; and WEISS, S. The role of the venous system in circulatory collapse induced by sodium nitrite, *J. Clin. Investigation*, **16**:85-91, 1937.
 287. WILSON, H. D. The relation between rhythmic variation in blood pressure and rhythmic contractions of the artery of the ear of rabbits and dogs, *Am. J. Physiol.*, **116**:295-301, 1936.
 288. WOODBURY, R. A., and AHLQUIST, R. P. The cutaneous vasodilating action of pitressin, *J. Pharmacol. & Exper. Therap.*, **86**:14-21, 1946.
 289. WOOLLARD, H. H. The innervation of blood vessels, *Heart*, **13**:319-36, 1926.
 290. WYBAUW, L. Transmission humorale de la vaso-dilation provoquée par l'excitation du bout périphérique des racines postérieures lombaires chez le chat, *Compt. rend. Soc. de biol.*, **123**:524-28, 1936.
 291. ———. Contribution à l'étude du rôle vasomoteur et trophique des nerfs sensitifs. I. Les vasodilatateurs (antidromiques) et le mécanisme de leur action, *Arch. internat. de physiol.*, **46**:293-323, 1938.
 292. ———. Contribution à l'étude du rôle vasomoteur et trophique des nerfs sensitifs; mécanisme périphérique de la vasodilatation antidromique, sa médiation chimique, *ibid.*, pp. 324-44.
 293. ———. Contribution à l'étude du rôle vasomoteur et trophique des nerfs sensitifs; les réflexes axoniques vasodilatateurs; leur signification fonctionnelle, *ibid.*, pp. 345-88.
 294. YATER, W. M., and CAHILL, J. A. Bilateral gangrene of feet due to ergotamine tartrate used for pruritus of jaundice; report of case studied arteriographically and pathologically, *J.A.M.A.*, **106**:1625-31, 1936.
 295. ZWEIFACH, B. W. The structure and reaction of the small blood vessels in Amphibia, *Am. J. Anat.*, **60**:473-514, 1937.
 296. ———. The character and distribution of the blood capillaries, *Anat. Rec.*, **74**:475-95, 1939.
 297. ———. The structural basis of permeability and other functions of blood capillaries, *Cold Spring Harbor Symp. Quant. Biol.*, **8**:216-23, 1940.
 298. ———. Peripheral circulation, *Ann. Rev. Physiol.*, **10**:225-58, 1948.
 299. ———. Basic mechanisms in peripheral vascular homeostasis, *Tr. 3d Conf. on factors regulating blood pressure*. New York: Josiah Macy, Jr., Foundation, 1949.

CHAPTER 5

Sensory Functions

I. THE SKIN AS AN ORGAN OF SENSES	121
A. The Four Fundamental Modalities	121
B. The Sensory Spots	121
C. Sensory Spots and Cutaneous Nerve Endings	123
D. Multiple Innervation of Sensory Spots	125
E. Spatial Discrimination	125
F. Sensory Units	126
G. Conduction of Sensory Impulses	126
H. Anatomical Pathways in the Central Nervous System	128
I. Adaptation and Afterdischarge	129
II. PAIN AND ITCHING	130
A. Different Qualities of Pain and Double Pain Sensations	130
B. The Hyperalgesic State	131
C. Itchy Skin	132
D. Hyperpathia and Meralgia Paresthetica	133
E. Causalgia	134
F. Quantitation of Pain Sensation	134
G. Relation of Pain and Itching	136
H. Tickling	138
I. Experimental Itch Stimuli	138
J. The Scratch Reflex	139
K. Itching and Vasomotor Reactions	140
L. Physiological or Minimal Pruritus	140
M. Itching in Pathological States	140
N. Chemical Mediation of Itching and Pain	142
O. Itching and Local Anesthetics	143
P. Pain, Itching, and Electrolyte Shifts	143
Q. Itching of Central Origin	143
R. Symptomatic Relief of Itching Excitability in Man	144
III. THERMAL SENSITIVITY	145
IV. TOUCH AND PRESSURE SENSATIONS	146

BECAUSE this book deals primarily with the skin, in this chapter the main emphasis will be on the peripheral events resulting in cutaneous sensations. The complex problems of central perception are beyond the scope of this book. Interested readers are referred to reviews by Dallen-

bach (29), Boring (23), Bishop (19, 20), Edwards (34), Beecher (10), Wolf and Wolff (144), Hardy, Wolff, and Goodell (60), Sperry (119) and, for the more philosophical aspects, to the recent monograph and bibliography of Hayek (62). Pain and itching have been dealt with in greater detail.

I. THE SKIN AS AN ORGAN OF SENSES

Sensory nerve endings in the skin represent mechanisms which transform environmental energies into nerve impulses. If the nerve impulse reaches the level of consciousness in the brain, a sensation is experienced. It was thought for a long time that impulses from single specific nerve endings are carried by single fibers to certain spots in the cortex, arousing there specific sensations with sharp temporal and spatial discrimination. Actually, the events are much more complicated.

A. THE FOUR FUNDAMENTAL MODALITIES

The skin mediates four main modalities of sensation, namely, pain (including pricking and burning pain, itching, and possibly tickle), touch (including light touch and pressure), cold, and warmth. The difficulties in accepting the concept of separate modalities as the functions of specified endings along special pathways to specified areas of the cortex have been admirably discussed by Bishop (19, 20). The statement on four modalities means only that "centrally, the ability exists to accurately differentiate subjectively, *at least* four sensations in simple experimental situations: and peripherally, *at least* four corresponding classes of endings can be inferred . . . to different and characteristic forms of energy." This qualifying statement on the four modalities is necessary because there are more than four types of endings in the skin and because various types of stimulation of a single sensory spot may arouse different sensations. While it is true that stimulation of cold spots will elicit the sensation of cold, no matter how they are stimulated, this is certainly not the case with pain spots. The threshold activation of pain endings in the skin (16) or in the cornea (126) causes an "unpainful pain," which is a nondescript contact sensation. Furthermore, stimulation of the same endings can cause an itching sensation, depending on the intensity and type of stimulation and/or on the state of the periphery. Touch sensations, as well as pain sensations,

may behave differently when the endings are stimulated mechanically or electrically. Although the different endings are differentially sensitive to different forms of energy, they may respond to "nonspecific" stimuli—and often do. The final sensation can be, and probably is, modified by events at the synapses and in the subcortical and cortical centers. There are complex sensations which are due to combinations of several simultaneous impulses of the same or different modalities, such as sensations of roughness, smoothness, and wetness. Rapid repetitive stimulation of touch endings elicits the sensation of vibration. Still the concept of basic modalities has retained its usefulness and has an objective basis, since ascending pathways within the spinal cord segregate roughly according to modality (129, 104).

B. THE SENSORY SPOTS

For all modalities, the skin surface consists of a mosaic of highly sensitive points, each surrounded by a progressively insensitive zone. This spotlike distribution of cutaneous sensations, classically established by the work of von Frey (38), has often been confirmed (16, 17).

Touch spots, when mapped out on the human forearm, are irregularly shaped areas up to 5 mm. in diameter (20). The spots vary in size regionally and are smaller over areas of more acute discrimination (20). The size of the touch spots determines the limit of two-point discrimination; within one spot two simultaneous stimuli are not differentiated (16). Some spots are about equally sensitive all over, but others have a central point which is more sensitive than its periphery; the sensitivity of such spots is usually related to encapsulated endings. Their thresholds are lower than those of endings around follicles, possibly because of the deeper position of the latter in the skin (20).

Cold spots occur in groups of three to eight in the arm area, the groups spaced 10 mm. apart, the points within a group 1–2

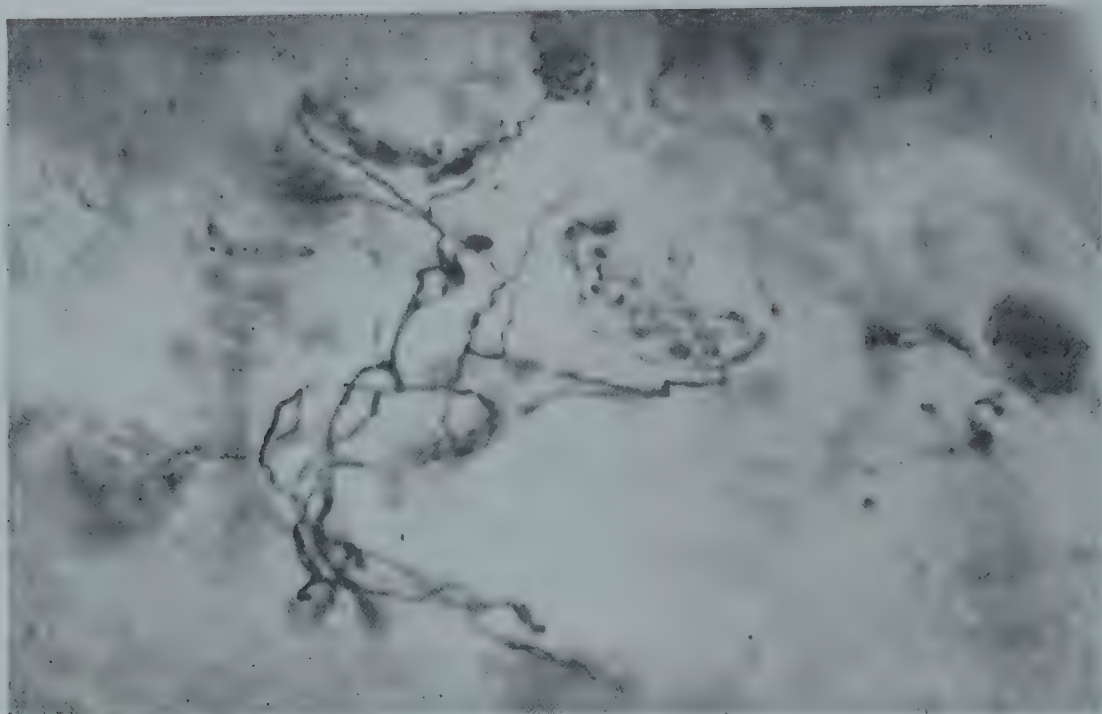


FIG. 1.—Photograph of a group of Krause's end-bulbs subserving cold sensibility. From Weddell (135). (Reproduced by permission of the author and the British Medical Bulletin.)

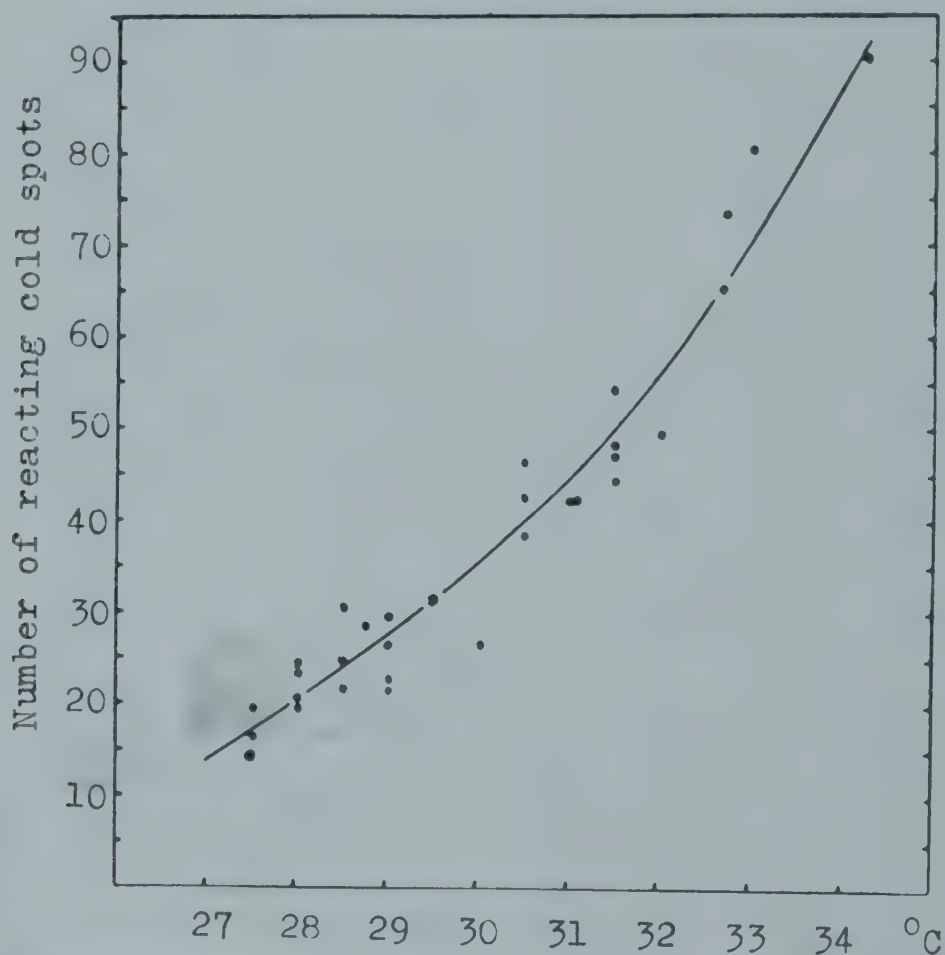


FIG. 2. —The number of reacting cold spots in a 6-sq. cm. skin area as a function of the skin temperature. From Bing and Skouby (14). (Reproduced by permission of Dr. Skouby and the *Acta physiologica Scandinavica*.)

mm. apart (20). The location of these spots corresponds well with the distribution of Krause's end-bulbs (Fig. 1). On the prepuce their average depth is about 0.1 mm. below the surface, and their average density is 15 per square centimeter (9, 7). Single cold spots in this area yield sensations when stimulated with a temperature decrease of as little as $0^{\circ}2$ C. produced at a rate of $0^{\circ}4$ C/sec. If a larger area is stimulated, even smaller changes in temperature, induced at a slower rate, can be perceived because of spatial summation (9, 7). Cold spots can be stimulated by mechanical, electrical, and even heat stimuli, the response always being a sensation of cold.

Warmth spots are not easy to map out. The difficulty probably arises from the fairly deep site of the end-organs. In the prepuce they are at a depth of 0.3 mm., their density is 1 or less per square centimeter (9, 7). Single spots can be stimulated by a temperature increase of $0^{\circ}3$ C. produced at a rate of $0^{\circ}2$ C/sec. Again, much smaller and slower changes suffice to cause warmth sensation if larger areas are stimulated. The degree of spatial summation was precisely calculated by Hardy and Oppel, who used radiating heat and cold (without contact) in their study of the temperature senses (52, 53). These authors' experiments indicated that temperature sensations do not depend on vascular changes in the skin; warmth and cold sensations can be elicited without any change in circulation. Interestingly, however, as shown in Figure 2, it seems that the number of reacting cold spots increases with increasing skin temperature (14).

Pain spots are distributed more densely than spots of other modalities. Itching is elicited from exactly the same spots as pain (40). Each pain spot has a central point of greatest sensitivity (20). Between the roughly hexagonal pain spots, blank spaces occur which are wholly insensitive to pain (20). Density of pain and itch spots and thresholds in different regions are discussed on page 137. Great density of the itch spots was reported in the perianal area by Longo (77) (Fig. 3). Differences in the distribution of

these spots may be a factor in the predilectional localization of itching skin diseases (p. 138).

C. SENSORY SPOTS AND CUTANEOUS NERVE ENDINGS

The attempts to assign specific sensory modalities to specific nerve endings have culminated in the work of Wollard, Weddell, and their associates (131-37, 149-52), who combined histological observations with partial degeneration experiments in animals and in patients with dissociated sensory disturbances. Their findings are schematically represented in Figure 4. As already indicated, cold sensitivity is probably mediated by Krause's end-bulbs. Ruffini's end-

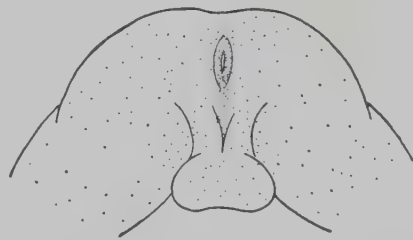


FIG. 3.—Relative density of itching points in the male anogenital region. From Shapiro and Rothman (109). (Reproduced by permission of the Williams & Wilkins Company.)

ings are probably the receptors for warmth. Within the modality of tactile sensations two subtypes were distinguishable: touch and pressure. Meissner's corpuscles in the corium, Merkel's disks about hair follicles and nerve terminals around the sheaths of hairs subserve the sensation of touch; Pacinian corpuscles and Golgi-Mazzoni endings, that of pressure. Finally, pain is mediated by free nonmyelinated endings of widely ramifying fibers which have a plexiform arrangement. The terminal axon ramifications spread over large areas and overlap with ramifications of other fibers. There is some disagreement about the localization of these free pain endings. Woollard (150), Bishop (20), and others find that the most superficial pain endings are subepithelial. In Woollard's (150) presentation they are particularly dense in the papillary body. However, from Weddell's findings (135) in an

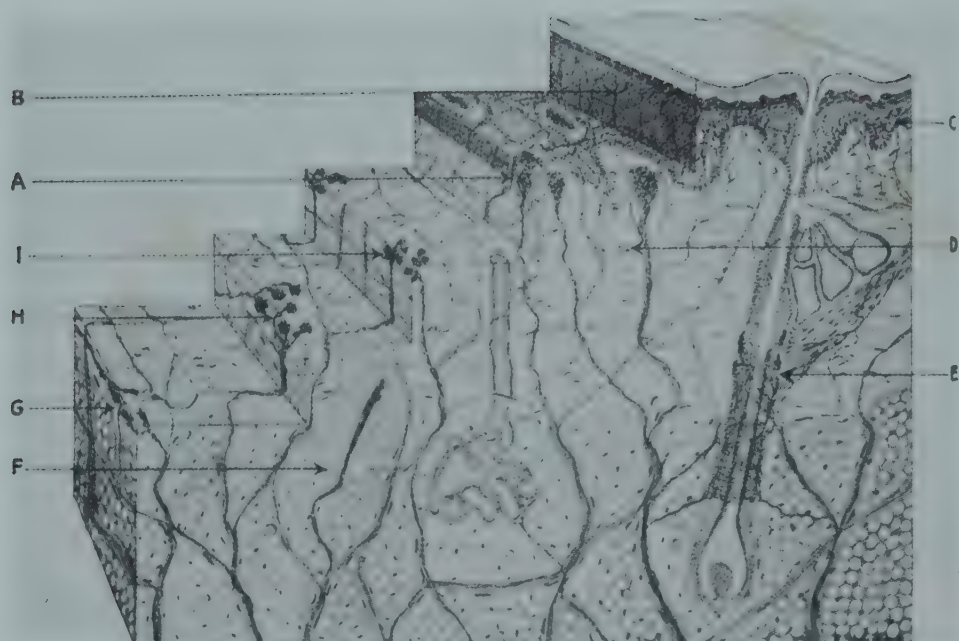


FIG. 4.—Cutaneous innervation. *A*, groups of Meissner's corpuscles subserving the sensation of touch; *B*, beaded nerve nets subserving pain (probably fast pain); *C*, Merkel's disks subserving touch; *D*, beaded nerve fibers derived from nerve nets subserving pain and associated with blood vessels (probably slow pain); *E*, nerve terminals around the sheath of a hair subserving touch; *F*, a Pacinian corpuscle subserving touch; *G*, a group of Ruffini endings subserving warmth; *H* and *I*, groups of Krause's end-bulbs subserving cold (these lie at somewhat variable depths beneath the skin surface). The organized endings are accompanied in every instance by fine-beaded nerve fibers subserving pain. From Weddell (135). (Reproduced by permission of the author and the British Medical Bulletin.)

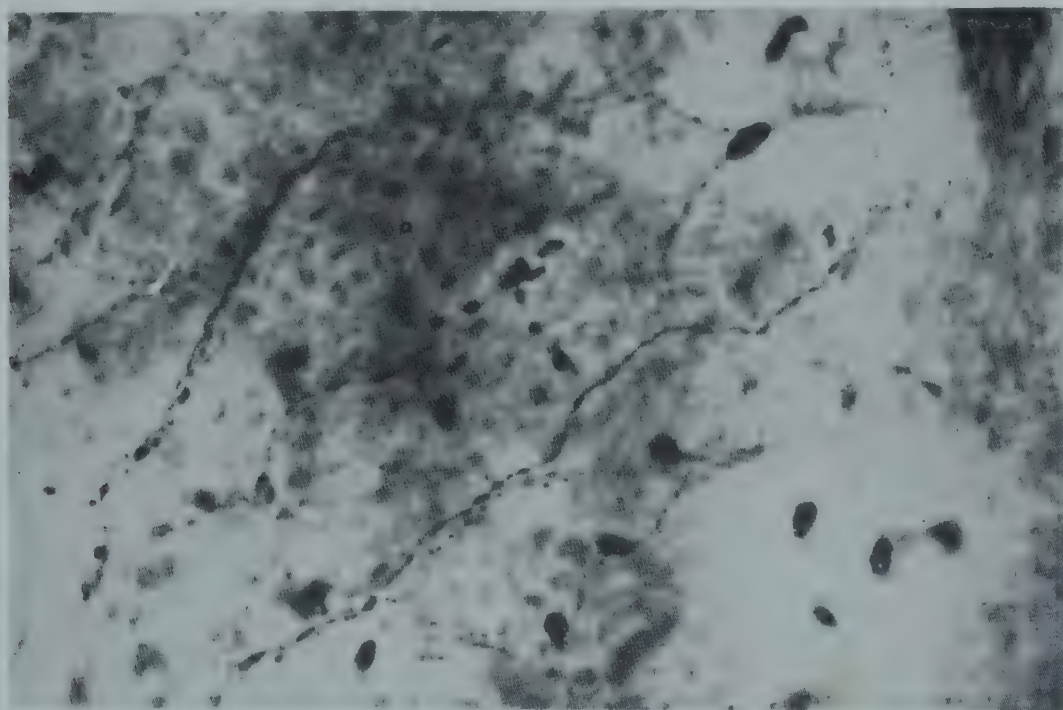


FIG. 5.—Photograph of beaded nerve nets subserving pain. Skin from a human foot. From Weddell (135). (Reproduced by permission of the author and the British Medical Bulletin.)

area where only pain sensations could be aroused, it appears that intraepidermal free nerve endings may mediate pain. In the snout of mammals, intraepidermal free nerve endings are particularly easy to demonstrate.¹

D. MULTIPLE INNERVATION OF SENSORY SPOTS

All fibers arising from cutaneous nerve trunks undergo extensive branching as they ascend in the skin. The arborizing fibers are arranged in meshwork patterns inclosing polygonal areas. The course of single fibers is rather difficult to follow, because the branches turn, describe loops, and inosculate with one another before reaching their end-points. However, the rami never fuse. There is no nerve "syncytium." Furthermore, branches of the same fiber traced through the ramifications in the plexus always terminate in similar nerve endings (135, 127).

1. There has been great reluctance on the part of many physiologists to accept the partial evidence which led to the assignment of specific endings to specific sensory modalities. The opposition has reached its climax in the recent work of Sinclair, Weddell, and their associates (D. C. Sinclair, G. Weddell, and E. Zander, The relationship of cutaneous sensibility to neurohistology in the human pinna, *J. Anat.*, 86: 402-11, 1952; E. Hagen, H. Knoche, D. C. Sinclair, and G. Weddell, The role of specialized nerve terminals in cutaneous sensibility, *Proc. Roy. Soc. s.B.*, 141: 279-87, 1953). The principal recent findings of these authors have been that the whole of the hairy skin in man, as contrasted with the glabrous skin, does not contain any organized endings. On the general body surface there are only two types of endings, viz., basket-like networks around the hairs and fine, profusely arborizing fibers which terminate freely. Still there is no appreciable difference in the sensory reception between hairy and glabrous skin or between hairy skin and adjacent mucous membranes, in which organized endings are present in profusion (e.g., lips). It is admitted that possibly in specified areas (palmar and plantar surfaces, nipples, genitalia) organized endings mediate specific modalities of sensation, but this cannot be the case for the skin in general. All previous pertinent data are questioned anyhow on the grounds that the histological criteria for identification of organized endings are vague. No definite morphological criteria can be set, for instance, for differentiation of Krause end-bulbs and Meissner corpuscles.

By using intravital stains and carrying out partial sectioning of nerves, Weddell (135) was able to follow the complicated course of sensory fibers in the skin. The main result of this study was that the single sensory spots have *multiple innervations from interlocking fibers approaching the same spot from different directions*. Figure 5 shows the interlocking of pain fibers in a skin area in which temperature and touch sensations were lost.

A good example of the extent of branching and multiple innervation is the situation in the rabbit's ear. Here the terminal branches of single fibers innervate hair follicles over areas as large as 1 cm. in diameter. Correspondingly, each fiber innervates as many as 300 follicle groups. Conversely, each follicle group is supplied by branches from at least two nerve fibers (131, 135), but usually by more.

E. SPATIAL DISCRIMINATION

The finding of multiple innervation of the unit sensory spots by fibers coming from all directions made it initially difficult to interpret the pointlike sharp localization of sensations in consciousness. T. Lewis (71, 72) represented most energetically the view that sharp spatial discrimination is incompatible with interlocking ramifying axon systems. He said that accurate location of sensation is not possible if the impulse enters "a system of branching axons connecting to a wide area of the skin" (71). I, too, thought that sharply localized prick sensations must derive from a single ending-fiber unit, while poorly localized itching might well arise in the widespread ramifications of pain fibers which mediate diffuse burning pain (97). However, the experiments of Weddell (135), Tower (126, 127), and Bishop (19, 20) led to the development of an interpretation practically opposite to the previous view, namely, that the power of localization and two-point discrimination depends on multiple innervation. If this multiple innervation is impaired, so that, for instance, instead of six or eight axon rami, all coming from different directions and originating in different

fibers, only one or two endings supply a single sensory spot, as is the case at the marginal overlap area after section of nerves, the sensations become poorly localized and faulty in other respects (135).

Tower's theory on spatial analysis (127) has completely changed the ideas on spatial discrimination. According to this theory, there is a central "pattern of excitation wherein fibers excited minimally encircle fibers more strongly excited." The most strongly excited fiber or fibers indicate the intensity of stimulation to the consciousness by a greater frequency of discharges (see below). The analysis of the pattern of simultaneous impulses of different strengths in the periphery makes it possible for the center to localize sharply. If the pattern of overlapping fibers is destroyed in areas of partial denervation, the basis for central spatial analysis is disordered, or even eliminated, because the cerebral cortex has to come to its interpretations on the basis of incomplete data. Spatial judgment then is less acute and often in error. If the anatomical unit of a single fiber with its widespread ramifications is isolated by removing all overlapping ramifications of other fibers, not only is the sharp localization of the sensation lost, but also the perception of intensity and the affect accompanying the sensation are abnormally acute (19).

F. SENSORY UNITS

From what has been said so far, it is clear that three varieties of so-called "sensory units" can be distinguished (19, 20, 104): (1) The anatomical unit which represents the terminal ramifications of a single fiber. These terminal ramifications overlap with others deriving from other fibers but do not anastomose with them. (2) The sensory spot, which is a surface area surrounded by zones which are not sensitive to the particular modality. It often has a central point of maximal sensitivity. According to Bishop (19, 20), this may be the point below which a nerve twig from the cutaneous nerve plexus turns up toward the surface of the skin. (3) The physiological unit, which is the

smallest area whose position on the surface can be accurately localized. This unit is variable regionally, and its extent depends also on the intensity, frequency, and sequence of stimulation.

G. CONDUCTION OF SENSORY IMPULSES

Brief and pointlike adequate stimulation of a single cutaneous sense organ elicits a rapid succession of numerous impulses in the nerve paths toward the center. The higher the intensity of the stimulus, the greater the number and/or frequency of the impulses. They can be demonstrated by electrical discharges which run along the nerves with the impulses.

The technic of amplifying and recording the action potentials of sensory impulses in peripheral nerves as used by Gasser and Erlanger (45, 36) led to the recognition of the basic fact that in a mixed nerve the conduction velocity of impulses varies with the fiber diameter (see also 21, 34). The larger the diameter of the fiber, the faster is its conduction. Thus fibers of different sizes contribute to distinct parts of the compound action potential of a mixed nerve, the wave of the fast-conducting, largest fibers showing up first and the wave of the smallest, slow-conducting fibers showing up last in the electroneurogram (Fig. 6).

The waves of action potentials obtained from cutaneous sensory fibers were classified, in the order of decreasing rate of conduction, as A (β , γ , δ , ϵ) and C waves. The β waves arise in myelinated fibers of more than 10 μ in diameter and have a conduction velocity of 30–60 meters per second. The C waves are impulses of thin nonmyelinated fibers with diameters below 5.5 μ and with conduction rates of 0.7–1.3 meters per second. The fiber diameters and conduction velocities of γ , δ , and ϵ waves are in between these two extremes. Each of them was referable to different maxima within the fiber-diameter spectrum. Fibers of different size differ also in regard to their threshold sensitivities and in their adaptation to continuous or repeated stimulation. Furthermore, there is a reverse behavior in their

susceptibility to asphyxia and to local anesthetics (72, 44, 19): impulses of fast-conducting, large fibers are the first to disappear on progressive asphyxiation of the nerve, while local anesthetics act first on slow-conducting, small fibers. By observing the order of disappearance of the sensory modalities under local anesthetics and/or asphyxia and correlating this order with the

the largest myelinated β fibers, although it may have representative components also in the δ group (19, 44). Two different kinds of touch impulses carried by fibers of different sizes, one arising from hair follicles, the other from the skin in between the follicles, were found by Bishop (16). This finding corresponds to two different kinds of touch receptors. Pain is mediated by at least two

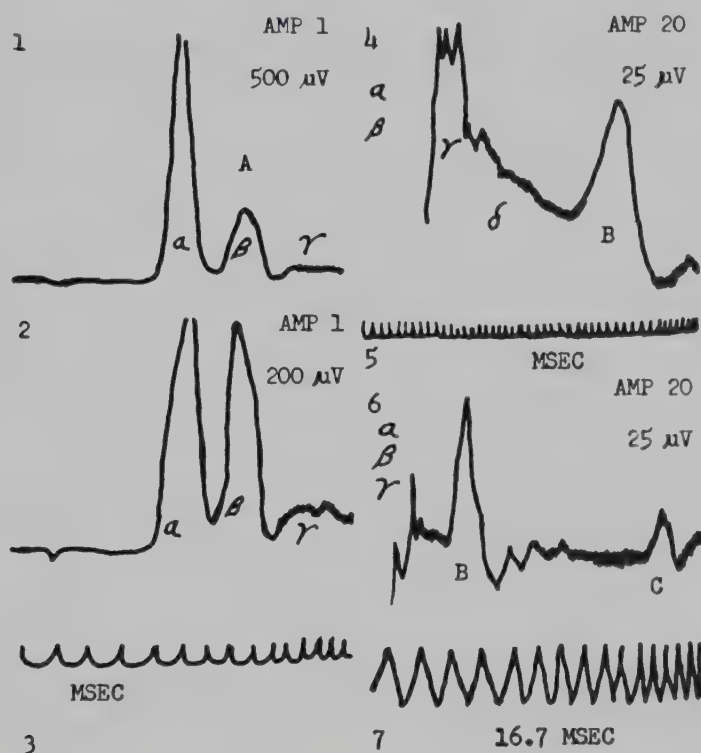


FIG. 6.—Complete action potential of bullfrog's peroneal nerve; distance of conduction 13.1 cm. The time falls off logarithmically from left to right; in 3 and 5 the period of the larger oscillations is 1 millisecond; in 7 the oscillation rate is 60 per second; 3 applies to 1 and 2, 5 to 4, and 7 to 6. From J. Erlanger and H. S. Gasser, *Electrical signs of nervous activity* (Philadelphia: University of Pennsylvania Press, 1937).

disappearance of special waves from the compound axon potentials, and also under a great many other ingenious experimental arrangements in partial nerve injuries, attempts have been made to correlate certain sensory modalities with impulses in certain peripheral fibers.

Fibers subserving different modalities have not been found to be restricted to a narrow range of fiber diameters (19, 44). Gasser (44) and Bishop (19) discussed the limitations of this approach. Broadly, it was found that, in general, touch is conducted by

different types of fibers, one at the head of the δ group, the other in the C range. These two sets, with different conduction velocities, were related to the phenomenon of double pain sensation and to the different characteristics of first (sharp) and second (dull) pain (p. 130) (97, 104). However, Bishop emphasized that by electrical stimulation of the same pain spot both types of pain (pricking and burning) can be elicited according to the type of stimulation (single or repetitive electrical shocks) (19). Temperature sensations are mediated by δ fibers,

smaller than the δ pain fibers but overlapping with them. Temperature sense has not been excluded from the C group (19).

Impulses in afferent fibers do not necessarily reach consciousness. Bishop emphasized—and it is obviously important for dermatopathology—that impulses may end

impulses are transmitted (possibly through the mediation of one or more intercalated neurons) to neurons of the second order, with their cell bodies in the posterior horn and their axons promptly crossing to the contralateral side, then ascending in the anterolateral (spinothalamic) tract. They

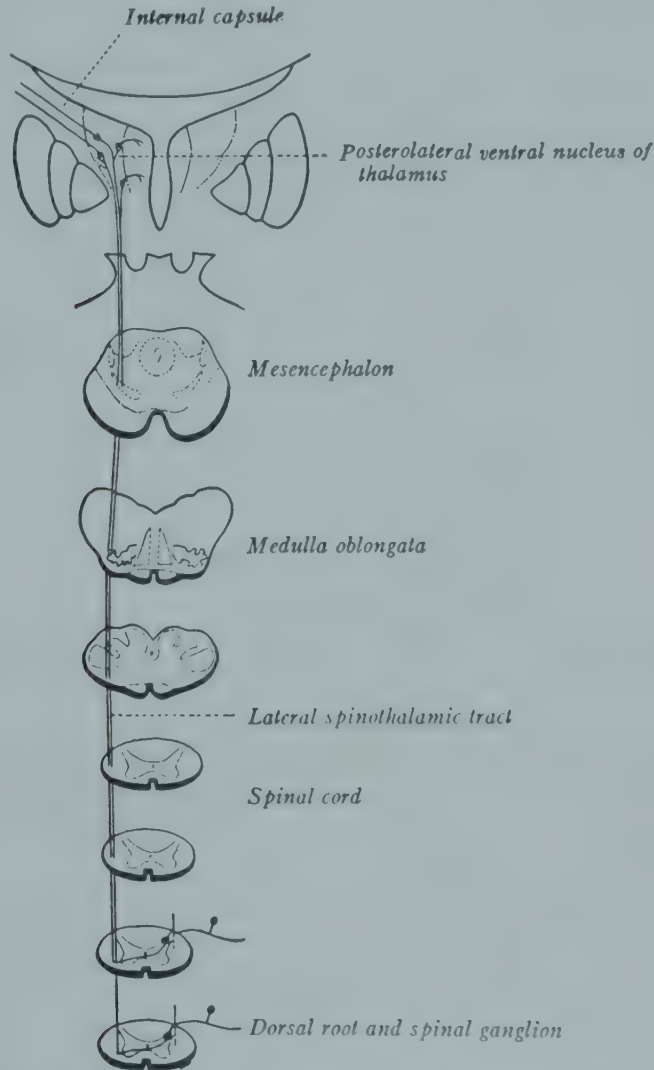


FIG. 7.—Diagram of the path for pain and temperature sensations. From Ranson (87). (Reproduced by permission of W. B. Saunders Company.)

up at reflex levels. They may elicit motor or secretory responses without being perceived.

H. ANATOMICAL PATHWAYS IN THE CENTRAL NERVOUS SYSTEM (87)

Pain and temperature impulses enter the cord through the central branches of the posterior-root ganglion cells (Fig. 7). This first neuron ends in the substantia gelatinosa in the apex of the posterior horn. Here the

end in the posterolateral ventral nucleus of the thalamus. Probably there also exists a chain of shorter neurons with frequent interruption in the gray matter of the cord. This chain ends at the same site in the thalamus as do the uninterrupted long axons. Neurons of the third order may lead from the thalamus to the somesthetic area of the cerebral cortex in the posterior central gyrus.

The central branches of the posterior-root ganglion cells of touch fibers enter the posterior funiculus and ascend there for some distance (Fig. 8). As they ascend, they give off collaterals to the gray matter at successive levels. The neurons of the second order arise in the posterior gray column at

patients with lesions of the anterolateral tract, while touch sensations remain intact (97).

I. ADAPTATION AND AFTERDISCHARGE

If touch, pressure, or temperature receptors are continuously stimulated with con-

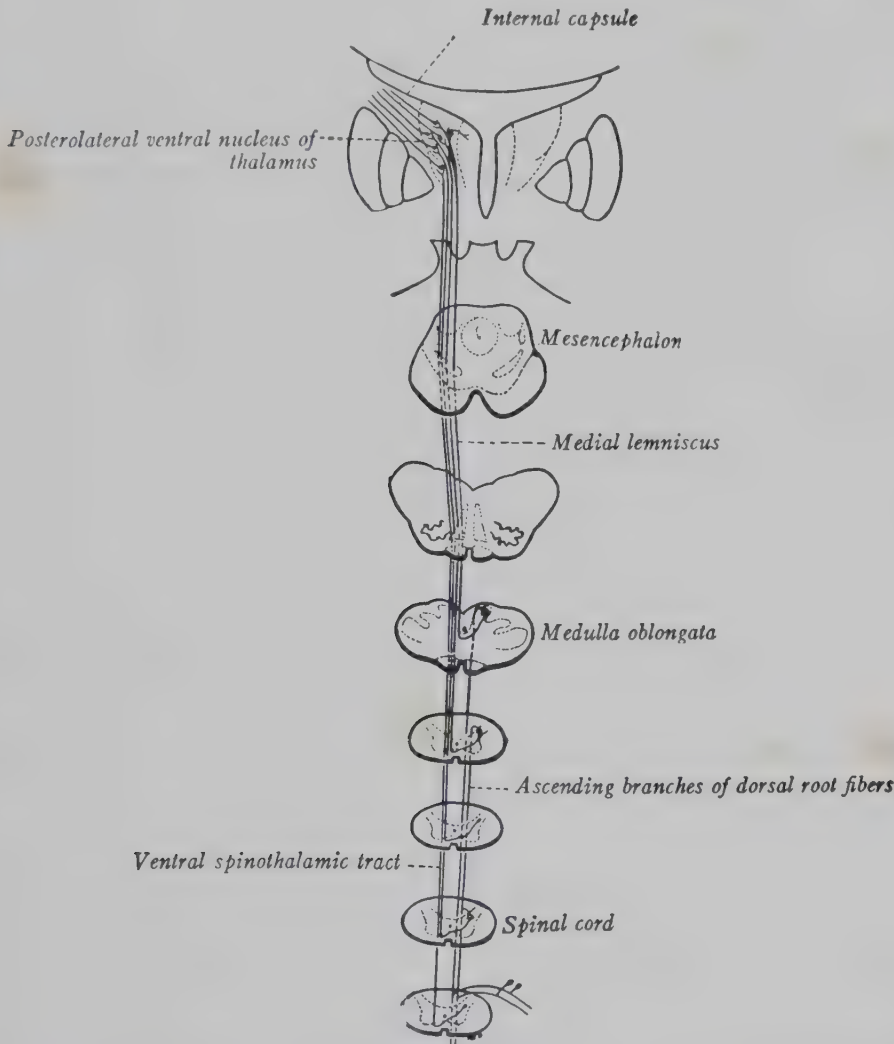


FIG. 8. Diagram of the tactile path. From Ranson (87). (Reproduced by permission of W. B. Saunders Company.)

these levels. Their axons cross the mid-line, form the ventral spinothalamic tract on the opposite side, and end in the thalamus. The neurons of the third order again end in the posterior central gyrus of the cortex.

Thus the central pathways of tactile sensations are clearly separate from those of pain (also itching) and temperature sensations. The latter sensations are absent in

stant intensity, the frequency of nerve impulses aroused gradually decreases, and eventually such impulses cease entirely. This phenomenon of adaptation is best illustrated by the relative ineffectiveness of stimuli with slowly but steadily increasing intensity and by the observation that a change in intensity of any stimulus is more effective than a steady state (19). Contact

with moderately warm water, for instance, elicits warmth sensation, but the sensation disappears in a few seconds when the temperature is kept constant. Both warmth and cold stimuli must have a critical rate of temperature change in order to be felt, otherwise adaptation nullifies the sensation.

Conversely, in some instances the activity of the sense organs continues after the stimulus is removed. This activity can be shown objectively by electrophysiological methods as afterdischarges.

Different types of sensory endings can be differentiated on the basis of differences in adaptation and afterdischarge. Touch receptors adapt with extreme rapidity and have no afterdischarge. The "on-off effect" in the touch sense is similar to that of some retinal endings (2, 18). The receptors would be unable to convey the sensation of vibration if they were not "dead-beat" (19, 104). Pressure receptors adapt considerably more

slowly than do tactile receptors. It seems to make a difference whether the inciting stimulus is deformation with or without pressure. The least adaptive endings are the pain receptors. They either adapt extremely slowly or even behave conversely, showing augmentation of the discharge with continuation of the stimulus (2, 19). Some pain impulses have strong and long-lasting afterdischarges. It is obviously of great biological significance that there is no adaptation to pain sensation, pain being a danger signal in many instances.

Under experimental conditions, adaptability can be modified. For instance, deprivation of calcium reduces it (33). Adaptation to pain is profoundly disturbed in inflammatory conditions of the skin. As pointed out by Bishop (19), not only changes in the sensory unit itself but changes in the nonnervous tissues surrounding the endings may markedly affect the sensory functions.

II. PAIN AND ITCHING

A. DIFFERENT QUALITIES OF PAIN AND DOUBLE PAIN SENSATIONS

"Pain" in everyday language connotes a sensation which is followed by an emotional reaction and an attempt to withdraw from the stimulating agent. Sensations elicited from pain endings in the skin are not necessarily so intense as that. In the presentation of Bishop (16, 19, 20), quantitated electric stimulation of the same pain endings in different spatial and temporal arrangement runs a gamut of sensations from an indifferent contact sense, through itch and non-painful prick, to pricking pain and to ache with affective aversion. All these sensations are due to activation of the same neural system. They depend on the number of fibers activated and on the frequency and total number of impulses, as they reach consciousness from a given locus. There is an effective spatial summation: stimulation of adjacent pain spots summates the effect to a more intense sensation. Similarly, temporal summation modifies the character of sensa-

tion: the perception of the afterdischarge of one volley is continuous with the response to the next.

True cutaneous pain, which is objected to, has at least two easily differentiated qualities: (a) a bright, pricking pain, which is momentary, temporary, and well localized, and (b) a burning pain, which is diffuse. These two sensations were assigned to two different sets of fibers, one conducting fast, the other conducting slowly. In the study of double pain sensations it was found that the first pain is pricking, short, pointlike, and well localized and the second is burning or itching and more unpleasant in character (97).

Double pain sensations can be elicited from the extremities by single pinpricks. The two successive sensations are separated from each other by a sensationless interval. If the double pain phenomenon is due to the presence of two sets of fibers with different conduction velocities, the sensationless interval between first and second pain should

diminish with shortening of the distance from the locus of stimulation to the brain. That this is actually the case was shown by Lewis and Pochin (74). On stimulation of toes and fingers, the sensationless interval is longest and diminishes as the locus of stimulation approaches shoulders and hips, corresponding with shortening of the pathways of the impulses. On stimulation of shoulders or hips, the difference in conductivity rate of the two pain-conducting sets is not large enough to make two distinct sensations, and first and second pain become fused. Evidence for the existence of two separate pain-conducting systems (δ and C fibers) was presented by means of electrophysiological work (26, 43) and by separating first and second pains by asphyxia and by local anesthetics (73).

Although Bishop maintains that both pricking and burning pain can be elicited from the same pain spot by using different patterns of stimulation and although Lewis (72) denied that first and second pain have different qualities, a clear differentiation between the two qualities was recently again confirmed by Bigelow, Harrison, Goodell, Graham, and Wolff (13, 51).

The possible existence of two separate sets of pain fibers is important for the interpretation of the itching sensation. While previously I believed (97) that itching is a function of slow-conducting C fibers, in a newer concept (51) there are two qualities of itching due to activation of two different sets of pain fibers: (a) a sharp pricking, superficial, well-localized itch and (b), a diffuse, burning itch. In this view the two different itch qualities can be assigned to the two different pain-conducting systems. In spite of rather impressive evidence in the field of double pain sensations, the existence of two separate sets of pain fibers is still not universally accepted (130).

B. THE HYPERALGESIC STATE

Following injury to the skin by pinching, crushing, or any other painful stimulus in a small circumscribed spot, a widespread area of hyperalgesia develops in the surrounding

skin, in which needle pricks cause unusually intense and diffuse and long-lasting pain and touch stimuli elicit soreness. Hardy, Wolff, and Goodell (59) called this state around the site of noxious stimulation "secondary hyperalgesia," to distinguish it from the primary hyperalgesia which develops at the site of the injury.

Primary hyperalgesia, as exemplified by the sensory state in a patch of ultraviolet light erythema, is associated with considerable lowering of all thresholds to noxious and nonnoxious stimuli. In secondary hyperalgesia there is little or no change in sensory thresholds. However, stimulation at threshold and at higher levels causes more intense and longer-lasting sensations than in normal skin. The two kinds of increased excitability can be characterized as (a) increased *sensitivity* with lowered thresholds and (b) no change in threshold but increased *sensibility* to threshold and supraliminal stimuli. It appears that in the two phenomena different kinds of spatial summation occur (59).

In Lewis' presentation (71) the secondary hyperalgesia, if fully developed, fills the territory of the cutaneous nerve in which the stimulus has fallen and is due to the excitation of a widely arborizing peripheral axon system of "nocifensor" nerves, which in itself does not mediate pain or other sensations but is responsible for changes in pain perception, in addition to causing vascular reactions (p. 89). Recent work has made it clear that such a system not only has no anatomical foundation but also does not need to be assumed from the physiological point of view (127, 104). It was shown, furthermore, that secondary hyperalgesia and axon-reflex flare, both supposed to be functions of the nocifensor system, are not related to each other (59).²

2. This does not, however, exclude the possibility that there are two different kinds of posterior-root fibers: sensory fibers with purely afferent functions and vasodilator fibers causing antidromic vasodilatation and reflex flare carrying efferent impulses. Lewis might have had this distinction in mind when he established the concept of a "nocifensor" system (p. 89).

In the work of Hardy, Wolff, and Goodell (59) secondary hyperalgesia was found to correspond not with peripheral nerve-supply areas but with segmental dermatomes. Their experiments indicate that hyperalgesia is created only if the irritated or injured spot is connected with the central nervous system. In their interpretation the hyperalgesic state develops because a barrage of noxious impulses from the site of injury produces a segmental excitatory state in the

Many observers found that in hyperalgesic zones weak stimuli, such as touch, stroking, weak pinpricks, and even heat and cold, cause itching sensations. This is denied, however, by Graham *et al.* (51).

C. ITCHY SKIN

After the state of "itching hyperexcitability" was described (95), Bickford (12) was the first to distinguish clearly between "spontaneous itch" and "itchy skin." After

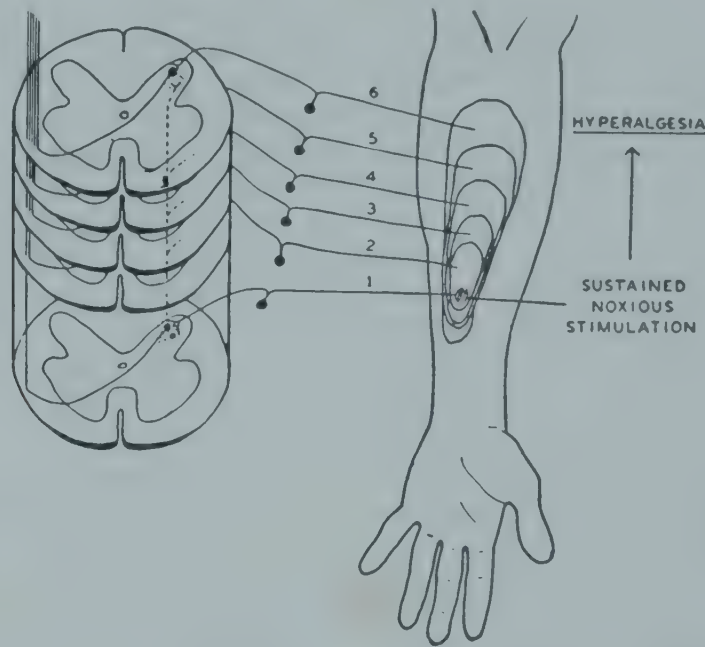


FIG. 9.—Diagrammatic representation of the relationship of a central excitatory state to cutaneous secondary hyperalgesia. From Hardy, Wolff, and Goodell (59). (Reproduced by permission of the American Society for Clinical Investigation.)

cord (Fig. 9). It is assumed that the afferent neurons enter a neuron pool in the dorsal horn and there make synaptic connections with a network of internuncial neurons in a manner like that described for motor pathways by Lorente de N6 (78). It is this neuron pool which is set into an excited state as a result of noxious stimulation of a small peripheral area. This concept, which transfers the site of the hyperalgesic state from the periphery to the cord, accounts for the type of spread of hyperalgesia and for the increased intensity and spatial summation of sensations without lowering of thresholds (Fig. 10).

the application of any stimulus which causes itching, two different phenomena occur: (a) there is an itching sensation confined to the point of stimulation, persisting for a short while after the stimulation has ceased, called "spontaneous itch"; (b) around the point of stimulation a widespread area can be mapped out which does not itch spontaneously but responds with itching to light friction and other "nonadequate" stimuli; this area is called "itchy skin."

Itchy skin and secondary hyperalgesia behave analogously. They appear alternately in the same region after more or less painful pointlike stimulation, and the zone of

hyperalgesia may be surrounded by a narrow zone of itchy skin (12, 51). Graham, Goodell, and Wolff (51) found that hyperalgesic and itchy zones are mutually exclusive: itching cannot be elicited from hyperalgesic zones; itchy skin is always hypoalgesic to pain stimuli.

As Lewis did for the hyperalgesic zone, Bickford (12) assumed a local axon-reflex activation in the periphery for itchy skin also, while Wolff and his associates (51) assumed that itchy skin, as well as hyperalge-

D. HYPERPATHIA AND MERALGIA PARESTHETICA

It might be an oversimplification to state that the two conditions of hyperpathia and meralgia paresthetica are extremes of hyperalgesia and of itchy skin, respectively, but most observations support such a statement. Hyperpathia is a most intense pain as it occurs in the so-called "thalamic syndrome," during recovery from peripheral nerve lesions and in some cutaneous scars, or it may be induced by prolonged compression of an

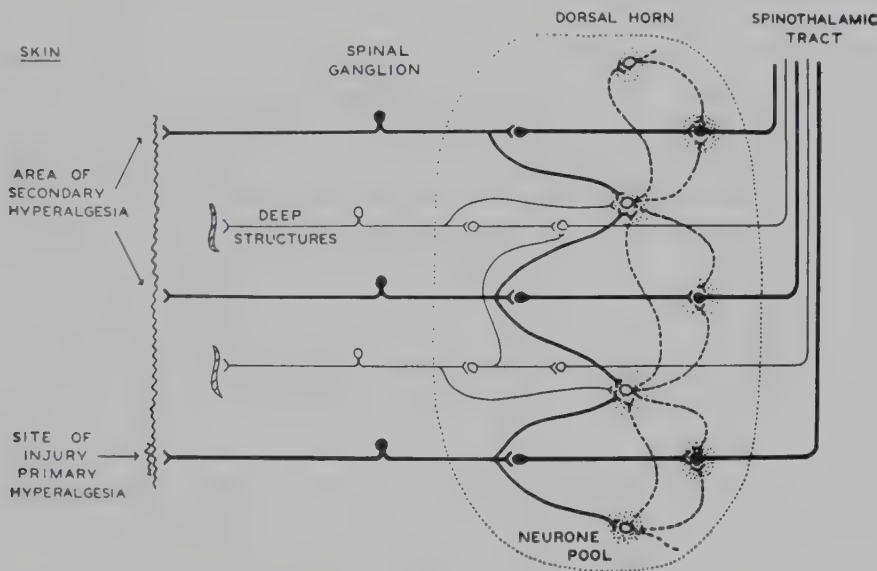


FIG. 10.—Schematic diagram of pain fiber connections within the neuron pool, showing foci of excitation (stippled areas) which result from the continuous barrage of noxious impulses from the site of injury. From Hardy, Wolff, and Goodell (59). (Reproduced by permission of the American Society for Clinical Investigation.)

sia, results from some activation of interneuronal neurons of the cord. The excitation of this system is above a certain intensity in hyperalgesia, and this is the reason that itching, which requires low-grade stimulation, cannot be elicited there. The finding of such exclusiveness is in agreement with some observations of Bickford (12) and with a remark of Pollock (85) that hyperpathetic responses (see below) never include itching. But they are not in agreement with the original description of the hyperalgesic state by Goldscheider (47, 48), who found most unpleasant itching after slight stroking in hyperalgesic areas; and they do not conform with my own experience.

extremity (80, 83, 115, 139). In this state, pain from a needle prick is felt not as a bright flash but as a slow, swelling, burning sting, with a withdrawal reflex hard to control and with autonomic reflex responses, such as sweating around the upper lip and contralateral palm and dilatation of the pupils. On testing pain thresholds, the skin in hyperpathic areas is found to be hypoalgesic. In hyperpathic scars, pain nets and terminals are isolated from their neighbors instead of interweaving with them, as is normal. It was postulated that the overreaction was caused by a reduction in normal pattern of impulses presented to the consciousness (139) (p. 126).

Meralgia paresthetica (105) is a disease marked by paresthesias in the outer surface of the thigh, which is supplied by the external cutaneous femoral nerve. The paresthesia consists of burning, tingling, stabbing pains of considerable severity or possibly only of a highly unpleasant numbness. The condition may be caused by traumatic nerve injury or by chronic pressure on the nerve from trusses, garters, etc. I had the opportunity to perform some (unpublished) experiments in such a case and found that the diseased area exhibited pronounced itching hyperexcitability more than any known example of "itchy skin." Punctiform itch stimuli, such as histamine punctures or application of a few grains of itch powder, cause a rapid spread of a most intense itching sensation over the whole diseased area, which lasts as long as the patient can keep himself from scratching.

E. CAUSALGIA (104)

Causalgia is a special case of hyperpathia in which vasodilatation complicates the picture. It appears after traumatic partial division of a peripheral nerve. The predominant symptom is severe burning pain, which the patient tries to counteract by keeping the area cold and wet. The pain is exacerbated by any stimulus, however slight, and also by anxiety or any emotional upset. There is no motor or sensory loss. The skin is tender, glossy, transparent, somewhat reminiscent of sclerodermatic skin in the early edematous phase. There are changes in the nails and in the tips of fingers or toes. Vasodilatation and sweating are associated.

In causalgia, sympathectomy at the appropriate level brings about complete relief in most cases. It has been assumed (75) that sensory neurons start a cyclic activity in internuncial neurons and that this activity initiates motor and sympathetic effects in the periphery, which further irritate the sensory system. Thereby a vicious cycle is created which can be terminated by sympathectomy. Doupe (31, 32) claimed that the cause for the cycle was direct excitation of sensory by sympathetic fibers either

at the site of the lesion or peripherally. He stated that the vasodilatation might be a result of stimulation of vasodilator sympathetic fibers (see p. 88).

F. QUANTITATION OF PAIN SENSATION

In the last twelve years Hardy, Wolff, and Goodell have dealt, in a series of careful and precise experiments, with the problem of quantitating cutaneous pain. By using a projected beam of heat and light, which stimulated a blackened area of the skin, they were able to express the intensity of the pain stimulus in physical terms and to elicit pure pricking pain with the exclusion of temperature and tactile impulses (56, 60). They found that the pain threshold for pricking pain, measured in this way, is remarkably constant in different individuals. With Schumacher (107), they studied a group of 200 subjects of both sexes, between the ages of ten and eighty, and reported an average pain threshold of 220 mcal/sec/sq cm, with a standard deviation of ± 7 mcal/sec/sq cm. They were able to raise the pain threshold for pricking pain considerably, by eliciting pain in other areas of the skin and by administering analgesics. Acetylsalicylic acid, although lowering the warmth threshold, was very effective in raising the pain threshold. However, in examining the effect of systemically administered analgesics, they found it necessary to dissociate pain perception from pain reactions. Opiates and ethyl alcohol, in particular, affected the reaction to pain much more than they did the perception of pain (146, 147, 148). Psychic suggestions interfered with the regularity of their results (145).

The authors succeeded in measuring just noticeable differences in intensities of pain sensations above the threshold level and were able to distinguish 21 steps between threshold and ceiling intensity of pain. They constructed a scale of pain intensity with units of the scale called "dols," each dol corresponding to two steps in noticeable intensity change (21 steps = $10\frac{1}{2}$ dols) (57, 58).

By prolonging the exposure time, the

authors could elicit and measure burning pain. But the establishment of thresholds and intensities for burning pain met greater difficulties than those for pricking pain (13, 34). This is regrettable, because for the analysis of hyperalgesic states and itchy skin alterations in burning-pain sensitivity are to be expected, rather than changes in pricking pain, since pure pricking pain (without afterdischarges) is hardly ever felt in such areas.

Possibly this is one reason why the examination of the sensitivity of itching and nonitching skin lesions with this method (86) did not yield much useful information. In this study there was a wider spread in threshold values than normal and some tendency for hyperalgesia in itching skin lesions. But there also was a number of lesions with pronounced hypoalgesia. It could not be established to what degree abnormal thickening of the horny layer contributed to raising the threshold in lesions with hypoalgesia. In the majority of itching skin lesions the pain threshold was not altered, and, with normal thresholds, the sensitivity to supraliminal stimuli was in some cases augmented and in others decreased. This study seems to prove the axiom that *the qualitative changes occurring in the sensitivity of damaged skin can hardly be characterized by quantitative measurement of the pricking-pain sensation.*

As far as quantitation of pain in general is concerned, the work of Hardy, Wolff, and Goodell was challenged in several publications by Beecher (10, 11, 61), whose main point was that perception of pain and the reaction to it vary immensely with the individual's attitude, momentary constellations, and suggestions. According to Beecher, constancy of pain threshold, pain perceptions, and pain reactions does not exist and cannot be expected; he particularly emphasized that it is impossible to evaluate analgesics with the methods proposed. Whyte (142) was unable to demonstrate the influence of aspirin and morphine on pricking-pain sensations elicited by radiant heat. The difficulty of quantitation is certainly

greatest in the field of burning pain and itching.

A new principle of estimating cutaneous sensory thresholds by the use of high-frequency square-wave currents was introduced by Sigel (112, 113).

Abnormal readiness to itch can develop in both hyperalgesic and hypoalgesic areas (97). While in some hypoalgesic states (for instance, during periods of increasing and decreasing local anesthesia) pain stimuli elicit itching (125), in hyperalgesia (at least in primary hyperalgesia) itching is easily provoked by light touch or stroking. This seemingly paradoxical behavior is consistent with the thesis that itching arises from weak stimulation of pain fibers. In hyperalgesia, subthreshold stimulation for pain, which normally causes only touch sensations, may elicit itching. And, vice versa, stimuli which cause pain in normal skin will cause only itching in hypoalgesic skin. Moreover, the actual pain thresholds in damaged skin probably depend to such a high degree on the extremely variable reactive thickening of the horny layer that one can hardly expect any regularity in their behavior in skin lesions. If pain threshold and pain sensitivity are measured by stimulating the endings with radiating heat, the influence of the horny-layer thickness is even greater than when testing is done with mechanical or electrical stimulation, because the latter stimuli break through the heat-insulating horny layer with greater ease.

A remarkable finding, however, obtained with radiating heat stimulation, concerns the influence of blood flow on thresholds. Schumacher (106) found no lowering of pain thresholds in pure arterial hyperemia, such as that elicited by nicotinic acid, but considerable lowering when the increased arterial blood flow was part of an acute inflammation, as is the case in ultraviolet light dermatitis. The increase in skin temperature was about the same in both instances. Thus it appears that it is not the increase in temperature but an additional factor in inflammation which affects the pain end-organs. A lowered pain threshold in areas of inflamma-

tion could be restored to normal by administration of acetylsalicylic acid. However, Schumacher's results are at variance with those of Weitz (141), who found a marked decrease in pain thresholds with increasing skin surface temperature. Thus it remains a debatable question whether itch thresholds under natural conditions are not influenced by increased blood flow to the skin. A number of empirical observations—for instance, a greater readiness to itch at the peak of diurnal temperature variations in the afternoon hours (p. 245) or under emotional stress connected with arterial hyperemia (p. 91)—strongly suggest that cutaneous blood flow has a great influence (see also the inhibitory effect of vasoconstriction, p. 140).

G. RELATION OF PAIN AND ITCHING

The evidence that itching sensations are elicited from the arborizing free pain-nerve endings close to the surface was summarized earlier by me (97, 98) and more recently by Graham, Goodell, and Wolff (51). The main line of proof is that itching can be elicited in areas with complete tactile anesthesia and that it is absent in analgesic areas with intact touch sense. Like pain, itching impulses are carried by the anterolateral tract in the cord (37). Itching is independent of the function of the temperature senses (97).

While there is almost complete agreement on the anatomical pathways of itching and also on the experimental evidence that itching arises when pain endings are weakly stimulated, there is much uncertainty as to how to explain the different qualities of the two sensations. Subjectively, weak pain and itching are easily distinguished, and, objectively, the motor responses—desire to withdraw in the case of pain and desire to scratch in the case of itching—are vastly different.

The earliest explanation for the different qualities was that itching arises if several pain endings are stimulated in specific spatial and temporal patterns (143, 40, 35). The same interpretation was given recently by Bishop (19, 20) who holds that itching is set up by rapid repetitive stimuli, each shock

being so weak that the summation of the effect of several shocks is needed to produce any sensation at all. He finds that itching corresponds to a slow, but persisting and uniform, frequency of fiber response.

There is no doubt that itching is produced with great ease when the stimulus is weak and repetitive and when several pain points are stimulated simultaneously. However, itching can also be elicited by pointlike single stimuli, mainly as an aftereffect of weak pinpricks (97) and particularly in the abnormal "itchy skin." Thus a particular spatial and temporal type of stimulation cannot be regarded as an absolutely obligate factor in the production of itching.

The second interpretation (97) was that there are two distinct sets of pain fibers, one mediating sharp, well-localized, bright, pricking pain (β fibers) and the other mediating a diffuse, more unpleasant, burning pain (C fibers). Under normal circumstances the latter sensation is suppressed or corrected by the former. But if by some injury or pathological event the sensitivity of the system mediating sharp pricking pain is impaired, the sensations of the second system are experienced in a pure form. When in such state the intensity of the noxious stimulus is gradually increased, superficial tickling sensation progresses to itching, and itching to diffuse burning pain, without critical change in the quality of sensation (153, 97). Itching is elicited when C fibers are weakly stimulated in the absence of δ -fiber excitation. Characteristically, the sensations mediated by C fibers persist after the cessation of stimulation, since the fibers are inclined to afterdischarges and their impulses are capable of central summation. They often appear as aftersensations of other sensory perceptions.

This interpretation was linked (97) with the assumption of Head (63) of two different—epicritic and protopathic—afferent systems, the first mediating sensations with sharp spatial as well as temporal, discrimination; the second mediating poorly or faultily localized sensations with an unpleasant diffuse quality. In areas with proto-

pathic sensitivity the thresholds are usually raised (hypoesthesia and hypoalgesia), but the sensations are intense and explosive at threshold levels.

The interpretation of itching as a weak protopathic pain, which I presented first some thirty years ago (94), was maintained in spite of the general consensus that Head's hypothesis of the two separate systems became untenable long ago (130, 97). The nomenclature of Head was adhered to because, regardless of its interpretation, the existence of "protopathic sensitivity" in nerve lesions and in itching skin lesions (95) has remained a fact, and reference is being made to it all the time (97, 55, 104).

Still the criticism that "no useful purpose" is served by retaining the terminology of Head (51) must be accepted, mainly because the work of Tower and Weddell (p. 126) made it clear that the "protopathic" type of sensitivity arises not when one system is eliminated, while the other becomes uninhibitedly prevailing, but when the multiple innervation of sensory spots by equal fibers becomes defective. Thus, according to the present concept, the "protopathic" quality of pain is produced not in an independent system of fibers but in the regular pain-conducting systems when the multiple innervation of sensory spots has been impaired. Such partial damage to fibers may be created experimentally or by morbid processes, and the damaged area acquires increased itching excitability (99). In my experiments (95) on itching skin lesions, I found in such areas that needle pricks caused abnormally intense, diffuse, radiating, and prolonged pain, with aftersensations of itching; touch stimuli elicited soreness; intense itching could be easily elicited by slight touch or by stroking and as an aftersensation, even when cold stimuli were applied. This is the state of "itching hyperexcitability" (95) or "itchy skin" (12) (p. 132).

In contrast to this concept of itching arising from defectively innervated endings of C fibers, the newest interpretation of itching (49, 51) implies that there are two types of itching sensations: (a) a sharp,

pricking, superficial, and well-localized itching, mediated by fast-conducting pain fibers, and (b) a burning itch, mediated by slow-conducting pain fibers. The experiments of Graham, Goodell, and Wolff (51) suggest that perception of burning itching depends on the presence in the cord of a circuit of internuncial neurons around which impulses travel constantly (Fig. 11). The impulses in this circuit are established by the low discharge frequency of weakly stimulated pain fibers. Impulses are sent from the circuit upward to the brain in an orderly fashion. The itch-producing stimulus is too weak to cause pain, but it does activate the nerve

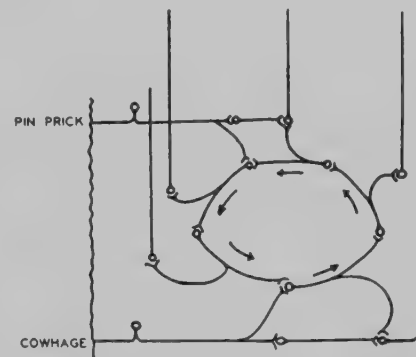


FIG. 11.—Suggested arrangement of circuits in internuncial neurons responsible for itching. Painful pinprick presumably breaks up the circuits. From Graham, Goodell, and Wolff (51). (Reproduced by permission of the American Society for Clinical Investigation.)

ending. Once itching has been produced, the pain thresholds are lowered because of this activation.

Little is known about regional differences of itching (and pain) excitability. Sigel (114) found low sensory thresholds on the face, bald scalp, and the instep. However, he used electrical stimuli, and, as he pointed out, in these areas the electrical resistance is low, so that the differences might have been due to greater accessibility of receptors to electrical stimuli in these regions. Probably the most valuable data are still those of von Frey (42). As shown in Table 1, he found the greatest density of pain points and low threshold values in the popliteal and cubital fossae, in the inguinal and jugular regions, and in the clavicular fossae, a dis-

tribution remarkably reminiscent of the predilectional sites of atopic dermatitis.

Itching excitability is certainly great around the large openings at the site of transitions between skin and mucous membranes. For the anal area of men this was

Bishop's (19) experiments also suggested that tickle was a form of touch. It requires an intermittent stimulus or stimulation of adjacent points and stimulation below a certain intensity. Movement of the stimulus was found to be an essential factor. In contrast, however, in most of the recent publications the sense of tickle is assigned to the pain system (97, 51), and Hardy *et al.* emphasize again the close relation of tickle to itch. In their experience tickle evokes the desire to scratch (59).

TABLE 1
DENSITY AND THRESHOLDS OF THE PAIN SPOTS IN DIFFERENT PARTS OF THE BODY (42)

SKIN REGION	PAIN SPOTS	
	Number/ Cm ²	Average Threshold (Gm.)
Scalp.....	144	1.35
Forehead.....	184	0.53
Dorsal aspect of nose.....	108	0.95
Tip of nose.....	44	1.00
Chin.....	196	0.55
Cheek.....	180	0.58
Lobe of ear.....	168	0.48
Anthelix of ear.....	136	0.57
Outer edge of ear.....	76	0.78
Eyelid.....	172	0.18
Hyoid region.....	204	0.48
Jugular region.....	228	0.26
Clavicular fossa.....	212	0.28
Nape of neck (hairline).....	176	0.65
Upper arm, medial aspect.....	208	0.29
Upper arm, lateral aspect.....	200	0.46
Cubital fossa.....	224	0.27
Forearm, volar aspect.....	203	0.32
Forearm, dorsal aspect.....	196	0.51
Dorsum of hand.....	188	0.66
Knuckle of thumb.....	60	2-4.0
Inguinal region.....	220	0.38
Thigh, anterior aspect.....	192	0.66
Thigh, posterior aspect.....	176	0.60
Popliteal fossa.....	232	0.30
Leg, anterior aspect.....	172	0.70
Leg, posterior aspect.....	160	0.56
Dorsum of foot.....	168	0.68
Sole of foot.....	48	2-4.0

I. EXPERIMENTAL ITCH STIMULI

In normal skin, the itching sensation can be elicited by mechanical, electrical, and chemical stimuli for experimental purposes. In the case of mechanical or electrical stimulation, certain intensities and spatial and temporal patterns facilitate the production of itching. Among chemical stimuli, dilute buffered solutions of histamine salts introduced by pinpricks, electrophoretically, or by intradermal injections are used most frequently, because histamine elicits itching in its purest form and because, by changing the concentration of the solution, the intensity of the sensation can be well graded. How effectively histamine can be used in studies on itching is exemplified in the work of Cormia (27), who was able to demonstrate diurnal variations of the itch threshold and decreased thresholds in pruritic lesions and after psychic trauma.

Itch powder—the powder and spicules from the plant *Mucuna pruriens*—probably acts by a combined mechanical and chemical effect, the spicules mechanically irritating the skin (55). After a short period of pricking and/or burning pain, the application of this powder to the skin causes pure itching. If the experiment does not require pointlike stimulation, itch powder is an excellent tool for experimentation (97, 51).

Because temperature and touch stimuli do not elicit itching in normal skin but may do so in altered skin, they usually are called “inadequate” or “nonspecific” stimuli of itch sensation.

As to whether itching has been elicited or

demonstrated by Longo (77) (p. 123). In some cases of brain tumors, itching of the nostrils was noted. In cats, itching (or at least the scratch reflex) can be most easily elicited from the external ear canal (25).

H. TICKLING

Tickling was often thought to be a function of touch receptors and touch fibers (97).

not, generally the unmistakable desire to scratch is used as a criterion in man. In animals the scratch reflex can be observed directly.

J. THE SCRATCH REFLEX

The motor response to itching is a spinal reflex (111). It is to some degree inhibited by the function of cortical centers (25). In human beings the different forms of the motor response to itching, such as scratching with the nails, rubbing or kneading the skin, are controlled to a certain degree by will power. How these movements alleviate itching is a difficult question to answer. My original view was that pain impulses of fast-conducting fibers were stimulated by scratching and that the sharp pain sensations suppressed itching, which is a function of the slow-conducting system. According to Bishop (19), itching comes about by low but persisting and uniform frequency of fiber responses; and scratching, inducing a new type of impulse in the same fiber system, breaks up the monotony of the fiber response without abolishing its activity. Similarly, for Graham, Goodell, and Wolff (49, 50, 51) scratching disturbs the regular rhythm of impulses in the circuit of internuncial neurons. These authors find that itching is promptly abolished by a pinprick, not only if it is applied in the itching zone but also if the prick is made at a considerable distance (up to 24 cm.) from the itching area (Fig. 11, p. 137). All that is needed to break up the pattern of itching impulses is to apply a new stimulus (of higher intensity?) within the same dermatome. This observation fits well the clinical experience that some patients with itching skin diseases pinch or knead the skin outside the itching area. Abolition of itching by pinpricks lasts longer than the prick sensation itself.

If, by scratching, the entire thickness of the epidermis is torn off with some of the connective tissue beneath, itching ceases immediately. This happens in cutaneous diseases either by tearing off elevated lesions (e.g., prurigo papules) or by excoriating the flat skin surface with "digging" movements

of the nails (e.g., neurotic excoriations). Otherwise, except if, by scratching, the itch stimulus is removed, scratching has only temporary effect. After the conditions for a return of itching have been restored, it may recur with a greater intensity, because the prick or scratch stimuli may have caused afterdischarges which may be felt as itching.

In pathologically increased itching excitability, scratching leads to a tormenting vicious circle, consisting of increasingly violent scratching and increasingly intensive itching. Such scratch paroxysms may last many hours and may cease only because of total somatic and psychic exhaustion of the patient.³

Scratch movements are usually rhythmic in man and animal. According to Sherrington (111), the rhythmicity is due to refractory periods in the spinal centers. In the modern view it appears that one pattern of rhythmicity of impulses excludes the other. Recently, Cornbleet (28) found that the average lengths of linear scratch marks become progressively shorter as one proceeds in the distal direction on the limbs. His findings suggested that there might be an inverse correlation between lengths of scratch marks and density of touch points. He assumed that, in order to suppress itching by scratching, a similar number of touch points must be stimulated everywhere on the body surface; wherever touch points are dense, scratch marks are short, and where touch points are far apart, the marks are long.

It has been an old clinical observation that in man, according to the nature of the pruritic skin disease, the technique of scratching (or rubbing) is different. In some diseases bloody linear scratch marks are common. In others, although they are extremely pruritic, such marks are not seen (101).

3. Discussion of the alleged relation of scratching to sexual instincts, scratching as a "cutaneous onanism" (64), "substitutive for sexual orgasm" (120), or indicating both erotic gratification and self-destruction (108), are beyond the scope of this presentation. For details see references 108, 103, 123.

K. ITCHING AND VASOMOTOR REACTIONS

In a skin area where the minute vessels have been constricted by a local injection of epinephrine, itching cannot be elicited (24, 81). It was concluded, therefore, that vasomotor reactions (which are inhibited by the capillary spasm in epinephrine anemia) are required for the elicitation of itching (24). While it is true that itching, particularly itching hyperexcitability, is often accompanied by vasodilatation or in some cases by vasoconstriction and piloerection, the available data suggest (96) either that vasomotor phenomena are co-ordinated with itching, both deriving from a common cause, or that they are a reaction to the sensory impulse coming from a reflex level, analogous to vasomotor reflex phenomena which follow pain sensations. It is probable that it is the local ischemia in the epinephrine experiment which prevents the impulse from arising in the appropriate endings: in the absence of sufficient oxygen, the sensitivity of pain endings is impaired (122). Interestingly, once itching has been induced, epinephrine injection does not abolish the continuous sensation. This observation possibly supports the assumption of a rather autochthonous circular excitation of neurons in the cord.

Itching is independent of sympathetic innervation. It can be elicited in sympathectomized areas as well as in normal skin, and the quality and intensity of the sensation are not different from normal. Still it has been claimed, on clinical grounds, that sympatholytic drugs, such as ergotamine, do decrease itching excitability (24). More recently this claim was indirectly supported by Königstein (67) in the case of a special experimental arrangement. By injecting "thalassin," an extract of tentacles of *Actinia*, intravenously into dogs and cats, he produced violent scratching spells in these animals. Administration of ergotamine tartrate prevented or aborted these spells.

Antidromic vasodilatation has not been shown to be necessary in eliciting the sensation of itching. The red flare of the triple response does not coincide with the zone of

hyperalgesia (59) or itchy skin. The administration of neither Mecholyl nor Banthine influences itch sensation (27).

Recently, it was reported that locally applied tetraethylammonium, a drug which blocks autonomic ganglia, prevents the development of itchy skin (118). No satisfactory explanation can be offered for this observation at the present time.

L. PHYSIOLOGICAL OR MINIMAL PRURITUS

"Physiological" or "minimal" pruritus are terms used to contrast the pruritus of pathological processes with the itching which is due to the weak stimuli of everyday life, such as slight rubbing, slight changes in pressure and temperature (e.g., in undressing). The impulses resulting from such stimuli are of minimal intensity and scarcely enter consciousness. However, a person may become conscious of this minimal pruritus when his attention is concentrated on it, as occurs in forced immobility, in boredom, and in fatigue (102). The instinctive activity of animals that is conducive to cleanliness was supposed to be caused by it (64).

No sharp limit can be drawn between physiological pruritus and itching which is elicited by pathological processes in the skin or in the nervous system. However, such a distinction is practicable if pathological pruritus is defined as itching evoked by morbid states and is of such intensity that it causes disturbances of well-being. The vicious cycle of itching-scratching paroxysms does not occur in physiological pruritus, although the latter is followed by short periods of scratching.

M. ITCHING IN PATHOLOGICAL STATES

From the clinical point of view, probably the most important result of experimentation in this field has been the distinction between "spontaneous itch" and "itchy skin."

It was stated (95, 96, 99) that "itching skin lesions," such as urticarial wheals, prurigo nodules, eczematiform vesicles, and lichenified lesions, itch "spontaneously" only during their development while irritat-

ing substances are being formed locally or carried there by the blood stream. These irritating substances are responsible for two independent phenomena: (a) the anatomic inflammatory lesion itself provoked by cell injury and (b) the itching provoked by stimulation of the nerve endings. This itching is of short duration, except in the case of continuous hematogenous transfer of irritating substances. However, in most instances, after the spontaneous itching has subsided, an increased itching sensitivity persists for many hours or even days, in the area of and around the lesions, regardless of whether the anatomic lesion has subsided, as in urticaria, or has not, as in the case of eczematous vesicles.

Even if the increased sensitivity is not very pronounced, as in some cases of urticaria, itching may always be elicited by stroking or rubbing, hours or days after the urticaria has subsided. This phenomenon is a part of what has been called somewhat poetically "mnemodermia," or "cutaneous memory" (64, 124). There is no itching skin disease in which it cannot be found.

In pathological degrees of itching hyperexcitability, all kinds of inadequate stimuli, such as light touch, light strokes, pressure, release of pressure, and temperature stimuli, elicit violent itch sensations. Although, in reality, this is a state of "itchy skin" or itching hyperexcitability, it is mistaken for a state of "spontaneous itch" because the slight and inadequate, but very effective, external stimuli escape notice (95, 96). In this state, one might say, even the slightest breath of air may evoke itching, and, under the conditions of everyday life, it becomes practically impossible to avoid all the trivial stimuli causing itch sensations.

It might be important to emphasize that it is not the microscopically visible anatomical change, the breakdown of cells, the accumulation of fluid, or cellular infiltrates which cause spontaneous itch. One out of two microscopically indistinguishable papules may itch while the other does not. The irrelevance of the anatomical change is best illustrated by the example of urticarial

wheals caused by insect bites. Itching starts before the wheal develops and disappears before the whealing has subsided (96). Thus it is clear that it is not pressure of the fluid in the upper corium which irritates the nerve endings. This view was confirmed with the advent of antihistaminic drugs: moderate suppressive doses of such drugs often prevent itching but not whealing.

In pathologically altered "itchy skin" I found (95) not only greatly intensified, radiating, and prolonged itching on adequate and inadequate stimulation but also intensification, prolongation, and radiation of pain sensations following needle pricks. This finding seems to be in disagreement with the new findings of Graham *et al.* (50, 51) on normal skin, indicating that hyperalgesia and itchy skin are mutually exclusive. Of course, pathologically altered skin may behave differently in this respect, although not necessarily. It is conceivable that in pathologically altered skin also there are high degrees of hyperalgesia in which itching cannot be felt because pain endings are so sensitive that even the slightest stimulus is too strong for creating such weak impulses as are required for itch elicitation. Such pathological states seem to be rare in dermatology. Possibly some phases of herpes zoster, which are not itchy at all at the height of hyperalgesia, belong here. In favor of mutual exclusiveness of pain and itch sensitivity, it could also be said that itching states are usually not truly painful, irrespective of the intensity of the process. Still, a severe contact dermatitis might occasionally cause burning pain, with no desire to scratch but with a tendency to withdraw from touch. Also, all sorts of oozing dermatitides may alternately itch or burn. The difference seems to be quantitative, and the clinician is familiar with transitory states. Possibly itching and pain hyperexcitability are mutually exclusive in secondary, but not in primary, hyperalgesia (pp. 133 and 135).

The main change in pathologically altered hyperalgesic and/or itchy skin seems to be a qualitative change of all sensations toward a diffuse, unpleasant, nondiscrimina-

tive, and explosive type. At present, this change can be best explained by impairment of the multiple innervation of sensory spots, which, in turn, may be caused by damage to the tissues around the endings or fibers (139, 138, 19, 20, 99).

Spontaneous itch often has a pricking character, as described by Brack (24) and Graham *et al.* (51), and for histamine wheals by Cormia (27). If itching is elicited in itchy skin by touch or stroke, the sensation usually lacks the pricking component. Thus one may speculate that the chemical substances which are formed during the development of itchy skin lesions stimulate both δ and C fibers (153), while itchy skin represents a hyperexcitability of C fibers only.

Practically speaking, one can assume that in some pathological pruritic states—for instance, in pruritus due to lymphoblastomas or uremia—spontaneous itch is present all the time because of the continuous transport of “toxic” substances to the skin, irritating the nerve endings and eliciting low-grade impulses. But in the great majority of pruritic conditions, such as urticaria, contact dermatitis, and atopic dermatitis, it is not the spontaneous itch but the state of itching hyperexcitability which creates the soil for the tormenting itching-scratching cycle.

The most classical example of pathologically increased itching excitability is lichenified skin. In what way this specific kind of thickened skin acquires the frightening state of abnormal sensitivity and promotes itching-scratching paroxysms is not understood. Possibly, investigations with Weddell's methods would shed some light on this mechanism.

N. CHEMICAL MEDIATION OF ITCHING AND PAIN

The theory of Thomas Lewis (69, 70) that itching and urticarial reaction are coordinated phenomena, both produced by release of “H-substance,” has been spectacularly confirmed by the advent of antihistaminic drugs. The generalization of Lewis' theory, however, that itching in all

instances is brought about by the release of histamine or a histamine-like substance, cannot be accepted because there is no histamine-induced itching without whealing. Whealing can be elicited with a very low concentration of histamine (1:1,000,000 or less), which still does not cause itching (97). This is the reason why small doses of antihistaminics relieve pruritus, while the urticaria may persist.

Claims for therapeutic effects of antihistaminics in itching conditions, other than those of the erythemato-urticarial group, are hard to evaluate because of the pronounced sedative side effects of many antihistaminics. The same is true for the itch-relieving effect of antihistaminics by local application: many of them have a local anesthetic effect independent of their antihistaminic action.

Not only in the domain of itching but also in that of pain sensation it was assumed that the stimulus does not act directly on the receptor, but indirectly by liberating a chemical agent from the surrounding tissue. Von Frey (39, 41) was the first to postulate such an event because of a frequently observed latent period in pain perception. After setting the stimulus, it may take several seconds until pain is perceived. Such a long interval could not be explained by slow conduction rates (117). Rein (89) assumed that there must be a pain-mediating chemical substance deriving from cell injury because, when pain is elicited by chemical agents, van't Hoff's law becomes applicable: a rise in temperature of 10° C. doubles the velocity of the reaction. In a series of publications Rosenthal (92, 93, 90, 91) attempted to present evidence that histamine is the pain-mediating agent. The difficulty in accepting Rosenthal's interpretation is two-fold. First, concentrations of histamine which cause pain are far above the physiologically occurring concentrations, and, second, cutaneous pain is not necessarily accompanied by whealing, which should be the case if histamine were responsible for pain (see above).

Recently, Bing and Skouby (15, 116)

demonstrated a considerable lowering in pain thresholds following the introduction of mecholyl. Skouby (117) found that simultaneous injection of acetylcholine and histamine produced distinct pain sensation when concentrations were used which separately did not evoke any sensation. Thus the possibility remains that in the presence of acetylcholine and by virtue of its "sensitizing" action, otherwise subthreshold concentrations of histamine may mediate pain sensations.

The possibility that pain sensation is mediated by liberation of potassium ions from injured cells was discussed by Bommer (22) and by Bárány (3). A "sensible substance" isolated from posterior roots (and not present in anterior roots) is held to be a chemical mediator by Umrath and Hellauer (128). This substance has vasodilator action but is not identical with acetylcholine (p. 90).⁴

O. ITCHING AND LOCAL ANESTHETICS

If it is true that itching is caused by weak impulses in pain fibers, local anesthetics should abolish it promptly because they paralyze pain sensations first. Actually, itching is prevented and/or abolished in analgesic skin (97). However, the practical difficulties in combating itching by local anesthetics are great. Most of the local anesthetics are potential allergens, their effect is temporary, and considerable concentrations are required to maintain perfect analgesia (110). To produce analgesia with topically applied anesthetics is difficult or impossible, because the penetration of most commer-

cially available water-soluble salts of local anesthetics through the intact skin is minimal, as contrasted with abraded skin and mucous membranes (55).

P. PAIN, ITCHING, AND ELECTROLYTE SHIFTS

Artificial introduction of potassium salts into the skin is exceedingly painful. An excess of potassium in the interstitial fluid was thought to be the cause of burning pain in wounds and in early phases of inflammation (22).

In a series of experiments Königstein (67, 68) demonstrated that in animals the intracisternal introduction of potassium salts elicits violent scratch paroxysms. Chemically related cations, such as cesium and rubidium, had similar effects. Ammonium salts, too, were effective, but their effect could be counteracted by a simultaneous injection of glutamic acid, which binds ammonia to form glutamine. By decreasing the calcium concentration in the cerebrospinal fluid by the addition of oxalate, phosphate, or citrate, Königstein also obtained scratch paroxysms. Intracisternal injection of calcium salts alleviated paroxysms, no matter whether they were elicited by "cationic shifts" or by intracisternal injection of alkaloids, such as morphine or pilocarpine. Magnesium was interchangeable with calcium in this effect. It cannot be decided whether scratch paroxysms actually indicate itch sensations, but they probably do. If so, in Königstein's experiments itching probably came about by central stimulation of itching pathways.

Q. ITCHING OF CENTRAL ORIGIN (97)

Centrally induced itching was observed in the cat after intracisternal injection of morphine (66, 97). After a violent scratch attack, itching hyperexcitability remained. This itching was purely central; it was projected also to areas where peripheral nerves were sectioned.

In human beings itching evoked without peripheral impulses has been observed in diseases of the central nervous system, such

4. Recently, in a careful series of experiments Armstrong, Dry, Keele, and Markham studied chemical excitants of pain by observing their sensory effects on the exposed base of a broken cantharidin blister in human skin. In this arrangement the following materials were found to produce pain and itching: acetylcholine chloride, potassium chloride, hypo- and hypertonic saline solutions, sodium citrate, acids below pH3, histamine salts, skin extracts, and the content of inflammatory blisters (D. Armstrong, R. M. L. Dry, C. A. Keele, and J. W. Markham, *Observations on chemical excitants of cutaneous pain in man*, *J. Physiol.*, **120**:326-51, 1953).

as tabes, dementia paralytica, and more rarely in manic-depressive psychosis, idiocy, and epilepsy. However, in human pathology purely central itching without any peripheral event seems to be altogether rare. However, the excitability of the sensory centers greatly influences the intensity of any kind of peripherally induced itching. If the itching is not very intense, the attention can be diverted from it (143). On the other hand, merely imagining the presence of biting insects may provoke itching and scratching in centrally hypersensitive individuals. This may be an accentuation of physiological (minimal) pruritus.

Pharmacologically, caffeine and the drugs of the benzedrine group intensify the central perceptibility of itching (96), while all the depressants of the central nervous system, except the opiates, have a depressing effect on itch perception. The effect of barbiturates, bromides, chloral hydrate, and acetylsalicylic acid are empirically well known but experimentally not quantitated.

Morphine and the related derivatives of opium, the most effective drugs for central relief of pain, do not decrease itching sensitivity but, on the contrary, usually increase it. In morphine-induced deep sleep, scratch movements can often be observed in patients with itching skin diseases. Morphine has an itch-provoking effect on the periphery and on subcortical centers (66). This behavior is reminiscent of the observation that thalamic pain is not alleviated by morphine. It is possible that depression of the cortex by opiates accentuates thalamic activity. The inhibitory effect of the frontal cortex on scratch movements was shown experimentally. If the inhibition is released, scratch movements become more violent (25). As mentioned before, scratch movements do not necessarily indicate itching, but they probably do.

R. SYMPTOMATIC RELIEF OF ITCHING EXCITABILITY IN MAN

Relief of itching excitability in man can be attempted by measures acting centrally and peripherally. Among centrally acting

agents barbiturates are probably most effective, because they act on the brain stem, where itching sensations are integrated. If itching excitability has been increased by conscious psychic conflicts (27), bromides acting on the cortex may bring relief (97). Acetylsalicylic acid and other antipyretics decrease the central perception of pain (144) and, as empirical experience indicates, of itching also. The use of chloral hydrate and paraldehyde is based merely on practical experience.

Peripheral measures can be classified in two groups. First, we try to keep the patient as free as possible from external stimuli which may start an itch attack. And, second, we attempt to decrease the state of hyperexcitability. Concerning the first point, if the patient is in bed, the bed linen should be smooth, light, and not too warm. If he is up, his clothing should be carefully chosen with a view to avoiding tight and roughly woven garments, especially woolen materials, which may irritate mechanically. Undressing always brings about a series of external stimuli, such as a sudden release of pressure and changes in temperature. Even persons with normal skin and with no itching hyperexcitability often experience itching when they undress, and they scratch for a while. The same situation leads to disastrous spells in itching patients, if the clothing has been too tight and if the room is too hot or too cold (98). Special precautions are observed in pruritus ani and vulvae in order to avoid any rubbing while cleaning the anus after bowel movements (109).

One may succeed partially in keeping away external stimuli by covering the skin with wet packs, lotions, liniments, pastes, and ointments. However, one has to choose a suitable type of application and supervise its removal and reapplication. The removing of old layers of ointments, etc., involves the danger of noxious stimulation and may start a new itching attack. Overdrying of lotions, pastes, and ointments is another danger. One has to know how fast external applications dry out on the skin and to time their change accordingly. Wet dressings have to

be changed every 5–10 minutes, lotions must be repainted on the skin every 4–6 hours; and pastes must be changed at least twice daily. Cleansing should be done with cautious patting movements, never with rubbing, and one should not attempt to remove the old layer completely (100, 99).

Attempts to decrease itching hyperexcitability often coincide with the attempts to treat the skin lesions symptomatically. In acute inflammatory processes, vasoconstriction by cooling application, wet dressings, and lotions brings about suppression of the inflammatory reaction and thereby decreases itchiness. In chronic dermatitis with lichenification any measure which reduces lichenification, such as X-ray irradiations or

application of tar preparations, will also reduce itching hyperexcitability. The lesion does not cause spontaneous itch but does cause itching hyperexcitability. The effect of X-ray irradiation on itching thresholds and itching excitability in lichenified skin was demonstrated quantitatively by Cormia (27). In normal skin such effects cannot be demonstrated (79).

In my experience the systemic administration of adrenocorticotrophic hormones and of cortisone does not decrease the intensity of itching sensation as long as the underlying disease is not markedly influenced. This experience is based on observations of patients with pruritus caused by malignant lymphoblastomas.

III. THERMAL SENSITIVITY

The assignment of cold spots to Krause's end-bulbs and that of warmth spots to Ruffini's cylinders (p. 123), as originally proposed by von Frey (42), is now accepted by many authors mainly on the basis of Bazett's work (4). His evidence has been that the number of Krause's end-bulbs and of Ruffini's cylinders is just about the same as the number of cold and of warmth spots, respectively, in a given surface are. However, direct proof of this relationship by mapping out cold and warmth spots and demonstrating the end-organs just below the spots histologically has not been presented (65, 82), with one remarkable exception. Weddell (135) found two groups of Krause's end-bulbs just below a cold spot which was marked by India ink on the human forearm.⁵ Glomus bodies (46) and free nerve endings (65) were also thought to mediate temperature sensations.

By measuring skin surface temperatures and temperatures in various depths of the skin by needle thermocouples on the forearm, Bazett and McGlone (6) were able to measure the thermal gradients within the

skin (Fig. 12). They found that the temperature of the skin rose sharply from the outer surface of the epidermis toward the upper part of the corium. The peak of temperature apparently corresponded to the superficial (subpapillary) arterial plexus. There followed a fall of temperature in the middle and lower corium, probably because of a scarcity of blood vessels there. There was a rise in temperature at the dermal-subdermal junction, probably coincident with the site of the deep vascular plexus. In flushed skin with the skin surface warmer, the gradient in the outer layer was greatly reduced, but the general shape of the curve of gradients was the same as in normal skin.

The relatively deep position of warmth receptors as contrasted with the superficial site of cold end-organs (see p. 123) was demonstrated by Bazett (4) on the male prepuce. He placed a thermocouple on one side of the prepuce and thermally stimulated the other side. By correlating changes in temperature of the skin with the time and the temperatures at which the subjects reported thermal sensations, the depth of thermal receptors could be well established.

Bazett (4) proposed the now generally accepted thesis that excitation of thermal

5. The significance of this single finding was questioned lately by Weddell himself and his associates when they stated it could have been due to mere coincidence (see n. 1, p. 125).

receptors occurs when the normal gradients of the skin are altered by thermal stimuli. Cold receptors are stimulated when the cold stimulus, by cooling the surface, causes an augmentation of the steep gradient in the superficial layers (Fig. 12). Warmth stimuli reverse this gradient by raising the surface temperature higher than the temperature at a depth of 1–3 mm.

Bazett's concept has been substantiated in many respects: (1) The latent period of warmth sensations is longer than that of

dient while the warmth stimulus must overcome and reverse the gradient. (3) Adaptation to warmth is more rapid than that to cold, because warmth receptors, lying deep, are more protected by vascular reactions. (4) Similarly, warmth spots when mapped on the skin surface are found to be less stable than cold spots, because they are more directly affected by vascular reactions (82). Still, as mentioned before, density of cold spots, too, may change considerably with changing skin surface temperature. In his

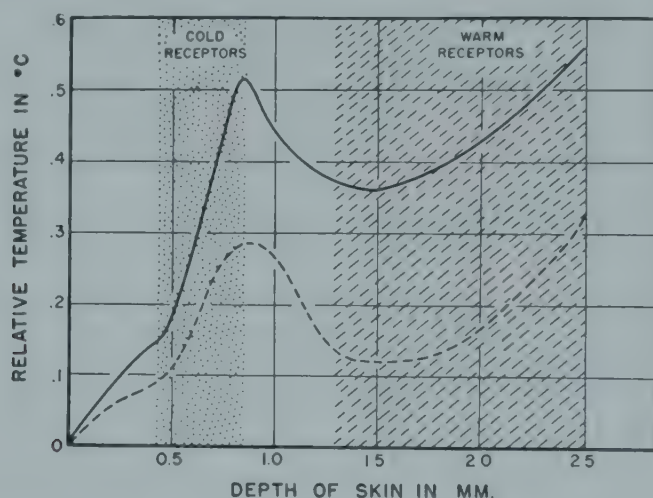


FIG. 12.—Thermal gradients of the skin. The solid line is for measurements taken when the skin was flushed with blood, and the broken line is for normal skin. The temperatures are the differences between the temperature of the surface of the skin and that of the skin at various depths. The probable positions of the cold receptors and warm receptors are indicated. Notice how they are related to thermal gradients. (By permission from *Physiological psychology* by C. T. Morgan and E. Stellar. Copyright, 1950. McGraw-Hill Book Company, Inc.)

cold sensations because of the greater depth of warmth receptors. (2) The threshold for cold sensation, as measured by temperature change increments, is less than that for warmth, because cold sensation is produced merely by exaggeration of the thermal gra-

latest work Bazett (5), in order to explain certain discrepancies in the mechanism of thermoregulation in response to external heat and to muscular exercise, postulated a second set of warmth receptors deep down at the dermal-subdermal junction (p. 248).

IV. TOUCH AND PRESSURE SENSATIONS

The main justification for attributing sensations of touch and pressure to special neural mechanisms, separate from those of pain, itching, and tickle, is their separate central pathway. Sensations of pain, itching, and tickle are absent in patients with lesions of the anterolateral tract, while touch sensations are intact (12). However, Bishop (19,

20) maintains that from the very same nerve endings nondescript contact sensations, "unpainful pain," itching, and frank pain can be elicited. Thus, whether the touch and pressure points are the only ones to mediate these sensations is still under discussion.

Von Frey (42) championed the idea of a

specific "sense of deformation" with a specific neural mechanism. He found that thresholds and intensities of tactile sensations depend on the degree and velocity of deformation. He insisted that there must be specific receptors (encapsulated end-organs) for transforming the deforming energy, because the threshold deformation stimulus for the touch sense involved about 10,000 times smaller energies than were required when the nerve was stimulated directly. In contrast, Bishop (20) found that free epidermal nerve endings do mediate touch sensation. In his presentation, deformation may elicit touch or prick or pain according to the curvature of the deforming object and thus according to the shape of the deformations.

Von Frey (42) emphasized that pressure and pull as well as decompression (recovery from the deformation) elicit identical sensations. Touch thresholds are dependent on adequate circulation, a finding more recently confirmed by Weitz (140, 141).

Topographically, Von Frey found that touch points could be assigned to each hair. He gave the following account (42): In young people the density of hair and of touch points is identical. Although with aging the number of hairs decreases, the loss of hair is not necessarily connected with loss of the sense organs. Nerve fibers reach the hair in two places: at the level of the hair papilla and at the narrowed neck of the shaft. Microsurgical experiments (121) proved that the papillary fibers are nonsensitive to touch and pressure. Therefore, the fibers around the neck of the shaft must be responsible. These fibers form a plexus which surrounds the glass membrane and enters the external root sheath. Some of these fibers end in cells of the character of Merkel's touch cells.

While touch thresholds do not vary much in different body areas, the density of touch points differs greatly according to region (42). There are 300 per square centimeter on the scalp and only 7 on the leg.

While the majority of touch receptors are

attached to nerve fibers around the hair, it is obvious that there must be receptors entirely independent of these appendages. Progressing distally on the volar aspects of the extremities to the nonhairy palmar and plantar surfaces, the density of touch points increases gradually and rapidly. Per square centimeter, Von Frey (42) counted 15 in the middle of the forearm, 40 on the wrist, more than 100 on the thenar, and found them so dense on the fingertips that counting became impossible. Meissner's tactile corpuscles were held by Von Frey to be the touch receptors of the nonhairy skin (42).

While it is true that these corpuscles occur mainly on the nonhairy parts, there are touch points also between hairs. Therefore, it is hard to understand von Frey's thesis that in hairy regions no other end-organs than those of the pilar system mediate touch. As a matter of fact, Bishop (16) found that the quality of touch sensation is different according to whether it is elicited from a hair or from the skin between follicles. Thus the equal numbers of touch spots and hairs in hairy skin as found by von Frey remain unexplained. Possibly, when von Frey minutiously established the thresholds of touch sensations of hairs, the interfollicular touch points with higher thresholds remained undiscovered. Hairs, acting as levers, intensify the deformation of the tissue and thereby may lower the threshold of sensation.

In the modern view (104), Meissner's corpuscles, nerve terminals around the sheaths of hair, and Merkel's touch cells are thought to mediate touch sensations, while Pacinian corpuscles and Golgi-Mazzoni endings are assigned to pressure. It seems that fine nerve fibers arising from the neurofibrillae of Meissner's corpuscles may pass into the epidermis and there form Merkel's disks (150), so that possibly these two touch organs are connected with each other and do not necessarily serve separate touch points. The role of the "hair disks," nerve elements discovered by F. Pinkus (84), in cutaneous sensations has not been investigated.

BIBLIOGRAPHY

1. ADRIAN, E. D. The mechanism of nervous action. Philadelphia: University of Pennsylvania Press, 1932.
2. ADRIAN, E. D., and UMRATH, K. Impulse discharge from Pacinian corpuscle, *J. Physiol.*, **68**:139-54, 1929.
3. BÁRÁNY, R. Untersuchungen über die durch subcutane und intramuskuläre Einspritzungen hervorgerufene aseptische Entzündung, gewisse Erscheinungen lokaler Allergie bei derselben und deren Bedeutung für die Physiologie und Pathologie, *Klin. Wchnschr.*, **11**:1852, 1932.
4. BAZETT, H. C. Temperature sense in man. *In*: Temperature: its measurement and control in science and industry, pp. 489-501. New York: Reinhold Publishing Corp., 1941.
5. ———. Theory of reflex controls to explain regulation of body temperature at rest and during exercise, *J. Appl. Physiol.*, **4**:245-62, 1951.
6. BAZETT, H. C., and MCGLONE, B. Temperature gradients in the tissues in man, *Am. J. Physiol.*, **82**:415-51, 1927.
7. ———. Sensation. II. The mode of stimulation of cutaneous sensation of cold and warmth, *Arch. Neurol. & Psychiat.*, **27**:1031-69, 1932.
8. ———. Sensation. III. Chemical factor in the stimulation of end-organ giving temperature sensations, *ibid.*, **28**:71-91, 1932.
9. BAZETT, H. C.; MCGLONE, B.; WILLIAMS, R. G.; and LUFKIN, H. M. Sensation. I. Depth, distribution and probable identification in the prepuce of sensory end-organs concerned in sensations of temperature and touch; thermometric conductivity, *Arch. Neurol. & Psychiat.*, **27**:489-517, 1932.
10. BEECHER, H. K. Perception of pain and some factors that modify it. *In*: ABRAMSON, H. A. (ed.), Problems of consciousness: transactions of the first conference, March 20-21, 1950, pp. 89-122. New York: Josiah Macy, Jr., Foundation, 1951.
11. ———. Experimental pharmacology and measurement of the subjective response, *Science*, **116**:157-62, 1952.
12. BICKFORD, R. G. Experiments relating to itch sensation, its peripheral mechanism and central pathways, *Clin. Sc.*, **3**:377-86, 1938.
13. BIGELOW, N.; HARRISON, I.; GOODELL, H.; and WOLFF, H. G. Studies on pain: quantitative measurement of two pain sensations of the skin, with reference to the nature of the "hyperalgesia of peripheral neuritis," *J. Clin. Investigation*, **24**:503-12, 1945.
14. BING, H. I., and SKOUBY, A. P. Influence of skin temperature on number of reacting cold spots, *Acta physiol. Scandinav.*, **18**:190-96, 1949.
15. ———. Sensitization of cold receptors by substances with acetylcholine effect, *ibid.*, **21**:286-302, 1950.
16. BISHOP, G. H. Responses to electrical stimulation of single sensory units of the skin, *J. Neurophysiol.*, **6**:361-82, 1943.
17. ———. The peripheral unit for pain, *ibid.*, **7**:71-80, 1944.
18. ———. The structural identity of the pain spot in human skin, *ibid.*, pp. 185-98.
19. ———. Neural mechanisms of cutaneous sense, *Physiol. Rev.*, **26**:77-102, 1946.
20. ———. The skin as an organ of senses with special reference to the itching sensation, *J. Invest. Dermat.*, **11**:143-54, 1948.
21. BISHOP, G. H.; HEINBECKER, P.; and O'LEARY, J. L. The function of the non-myelinated fibers of the dorsal roots, *Am. J. Physiol.*, **106**:647-69, 1933.
22. BOMMER, S. Neutralsalzreaktionen der Haut, *Klin. Wchnschr.*, **3**:1758-60, 1924.
23. BORING, E. G. Sensation and perception in the history of experimental psychology. New York: D. Appleton-Century Co., 1942.
24. BRACK, W. Die Bedeutung des vegetativen Systems für die Entstehung des Juckens. *In*: Delib. Ninth Internat. Dermat. Cong., **1**:129-67. Budapest: Inst. Typogr. "Patria," 1935.
25. BRADFORD, F. H. Ablation of frontal cortex in cats with special reference to enhancement of scratch reflex, *J. Neurophysiol.*, **2**:192-201, 1939.
26. CLARK, D.; HUGHES, J.; and GASSER, H. S. Afferent function in the group of nerve fibers of slowest conduction velocity, *Am. J. Physiol.*, **114**:69-76, 1935-36.
27. CORMIA, F. E. Experimental histamine pruritus, *J. Invest. Dermat.*, **19**:21-33, 1952.
28. CORNBLEET, T. Scratching patterns. I.

- Influence of site, *J. Invest. Dermat.*, **20**:105-10, 1953.
29. DALLENBACH, K. M. Pain; history and present status, *Am. J. Psychol.*, **52**:331-47, 1939.
 30. DOUPE, J. Studies in denervation. D. The mechanism of axonal vasodilation, *J. Neurol. & Psychiat.*, **6**:115-20, 1943.
 31. DOUPE, J.; CULLEN, C. H.; and CHANCE, G. Q. Post-traumatic pain and the causalgic syndrome, *J. Neurol. & Psychiat.*, **7**:33-43, 1944.
 32. DOUPE, J.; CULLEN, C. H.; and MACAULAY, L. J. Studies in denervation. F. The circulation in the skin of the proximal parts of the limbs, *J. Neurol. & Psychiat.*, **6**:129-32, 1943.
 33. DUN, F. T., and FINLEY, C. B. The rate of adaptation of cutaneous nerve endings in the frog, *J. Physiol.*, **94**:170-76, 1938.
 34. EDWARDS, W. Recent research on pain perception, *Psychol. Bull.*, **47**:449-74, 1950.
 35. EHRENWALD, H. Zur Pathologie und Klinik des Juckgefühls, *Ztschr. f. d. ges. Neurol. u. Psychiat.*, **132**:502-17, 1931.
 36. ERLANGER, J. The interpretation of the action potential in cutaneous and muscle nerves, *Am. J. Physiol.*, **82**:644-55, 1927.
 37. FOERSTER, O. Symptomatologie der Erkrankungen des Rückenmarks und seiner Wurzeln. *In*: BUMKE-FOERSTER, Handb. d. Neurol., **5**:1-403. Berlin: J. Springer, 1936.
 38. FREY, M. VON. Untersuchungen über die Sinnesfunktion der menschlichen Haut. I. Abhandl.: Druckempfindung und Schmerz. Leipzig: S. Hirzel, 1896.
 39. ———. Untersuchungen über die Sinnesfunktion der menschlichen Haut; Druckempfindungen und Schmerz, Abhandl. d. math.-phys. Cl. d. k. sächs. Gesellsch. d. Wissensch., **23**:169-266, 1897. Quoted by HARDY *et al.* (60).
 40. ———. Zur Physiologie der Juckempfindung, *Arch. néerl. de physiol.*, **7**:142-45, 1922.
 41. ———. Versuche über schmerzzerregende Reize, *Ztschr. f. Biol.*, **76**:1-24, 1922.
 42. FREY, M. VON, and REIN, H. Physiologie der Haut. *In*: JADASSOHN, Handb. des Haut- u. Geschlechtskr., **1/2**: 1-160. Berlin: J. Springer, 1929.
 43. GASSER, H. S. Conduction in nerves in relation to fiber types, *A. Res. Nerv. Ment. Dis. Proc.*, **15**:35-59, 1934.
 44. ———. Pain-producing impulses in peripheral nerves, *ibid.*, **23**:44-62, 1943.
 45. GASSER, H. S., and ERLANGER, J. The role played by the sizes of the constituent fibers of a nerve trunk in determining the form of its action potential wave, *Am. J. Physiol.*, **80**:522-47, 1927.
 46. GILMER, B. VON H., and HAYTHORN, S. R. Cutaneous pressure-vibration spots and their underlying tissues, *Arch. Neurol. & Psychiat.*, **46**:621-48, 1941.
 47. GOLDSCHIEDER, A. Über Irradiation und Hyperästhesie in Bereich der Hautsensibilität, *Arch. f. d. ges. Physiol.*, **165**:1-36, 1916.
 48. ———. Das Schmerzproblem. Berlin: J. Springer, 1920.
 49. GRAHAM, D. T., and GOODELL, H. Experimental observations on neural mechanisms involved in itching, *Fed. Proc.*, **8**:59, 1949.
 50. GRAHAM, D. T.; GOODELL, H.; and WOLFF, H. G. Itch sensation in the skin: experimental observations on the neural mechanism involved, *Tr. Am. Neurol. A.*, **75**:135-38, 1950.
 51. ———. Neural mechanisms involved in itch, "itchy skin" and tickle sensations, *J. Clin. Investigation*, **30**:37-49, 1951.
 52. HARDY, J. D., and OPPEL, T. W. Studies in temperature sensation. III. The sensitivity of the body to heat and the spatial summation of the end-organ responses, *J. Clin. Investigation*, **16**:533-40, 1937.
 53. ———. Studies in temperature sensation. IV. The stimulation of cold sensation by radiation, *ibid.*, **17**:771-78, 1938.
 54. ———. Stimulation of the cold end organs of the human skin by radiation, *Am. J. Physiol.*, **123**:89-90, 1938.
 55. HARDY, J. D.; POTEUNAS, C. B.; and MEIXNER, M. D. Pain threshold measurements on human skin following application of topical analgesics, *J. Invest. Dermat.*, **16**:369-77, 1951.
 56. HARDY, J. D.; WOLFF, H. G.; and GOODELL, H. Studies on pain. A new method for measuring pain threshold: observations on spatial summation of pain, *J. Clin. Investigation*, **19**:649-58, 1940.
 57. ———. Studies on pain. Discrimination of differences in intensity of a pain stimulus as a basis of a scale of pain intensity, *ibid.*, **26**:1152-58, 1947.
 58. ———. Studies on pain. An investigation of some quantitative aspects of the dol

- scale of pain intensity, *ibid.*, **27**:380-86, 1948.
59. HARDY, J. D.; WOLFF, H. G.; and GOOD-ELL, H. Experimental evidence of the nature of cutaneous hyperalgesia, *ibid.*, **29**: 115-40, 1950.
 60. ———. Pain sensations and reactions. Baltimore: Williams & Wilkins, 1952.
 61. HARDY, J. D.; WOLFF, H. G.; GOOD-ELL, H.; and BEECHER, H. K. Pain—controlled and uncontrolled, *Science*, **117**:164-67, 1953.
 62. HAYEK, F. A. The sensory order. Chicago: University of Chicago Press, 1952.
 63. HEAD, H.; RIVERS, W. H. R.; and SHERREN, J. The afferent nervous system from a new aspect, *Brain*, **28**:99-115, 1905.
 64. JACQUET, L. Troubles de la sensibilité. *In*: La pratique dermatologique, **4**:330, 1904. Quoted by ROTHMAN (96).
 65. JENKINS, W. L. A new basis for cutaneous temperature sensitivity. *In*: Temperature: its measurement and control in science and industry, pp. 502-8. New York: Reinhold Publishing Corp., 1941.
 66. KÖNIGSTEIN, H. Über Lokalisationsversuche des durch Morphinum ausgelösten "Kratzwerkes" im Centralnervensystem, *Arch. internat. de pharmacodyn. et de therap.*, **62**:1-13, 1939.
 67. ———. Experimental study of itch stimuli in animals, *Arch. Dermat. & Syph.*, **57**:828-49, 1948.
 68. ———. Shifting of cations in the cerebrospinal fluid as a cause of pruritus, *J. Invest. Dermat.*, **17**:99-123, 1951.
 69. LEWIS, T. The blood vessels of the human skin and their responses. London: Shaw & Sons, Ltd., 1927.
 70. ———. Clinical science illustrated by personal experiences. London: Shaw & Sons, Ltd., 1934.
 71. ———. Experiments relating to cutaneous hyperalgesia and its spread through somatic nerves, *Clin. Sc.*, **2**:373-423, 1936.
 72. ———. Pain. New York: Macmillan Co., 1942.
 73. LEWIS, T., and POCHIN, E. E. Double pain response of human skin to a single stimulus, *Clin. Sc.*, **3**:67-76, 1937.
 74. ———. Effects of asphyxia and pressure on sensory nerves of man, *ibid.*, **3**:141-55, 1938.
 75. LIVINGSTON, K. E. Pain mechanisms: a physiologic interpretation of causalgia and its related states. New York: Macmillan Co., 1942.
 76. ———. Phantom limb syndrome; nerve neuromas, *J. Neurosurg.*, **2**:251-55, 1945.
 77. LONGO, V. Sulla topografia della sensibilità del "prurito," *Arch. di fisiol.*, **36**:197-204, 1936.
 78. LORENTE DE NÓ, R. Analysis of the activity of the chains of internuncial neurons, *J. Neurophysiol.*, **1**:207-44, 1938.
 79. LORINCZ, A. L. Personal communication.
 80. MAGOUN, H. W. Somatic functions of the nervous system, *Ann. Rev. Physiol.*, **11**:161-72, 1949.
 81. MELTON, F. M., and SHELLEY, W. B. The effect of topical anti-pruritic therapy on experimentally induced pruritus in man, *J. Invest. Dermat.*, **15**:325-32, 1950.
 82. MORGAN, C. T., and STELLAR, E. Physiological psychology. New York: McGraw-Hill Book Co., Inc., 1950.
 83. PATTLE, R. E., and WEDDELL, G. Observations on electrical stimulation of pain fibers in exposed human sensory nerve, *J. Neurophysiol.*, **11**:93-98, 1948.
 84. PINKUS, F. Die normale Anatomie der Haut. *In*: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1/1**:1-378. Berlin: J. Springer, 1927.
 85. POLLOCK, L. J. Discussion on ROTHMAN (97).
 86. POTELUNAS, C. B.; MEIXNER, M. D.; and HARDY, J. D. Measurement of pain threshold and superficial hyperalgesia in diseases of the skin, *J. Invest. Dermat.*, **12**:307-16, 1949.
 87. RANSON, S. W. Anatomy of the nervous system. Philadelphia and London: W. B. Saunders Co., 1953.
 88. RANSON, S. W.; DROEGEMUELLER, W. H.; DAVENPORT, H. K.; and FISHER, C. Number, size and myelination of the sensory fibers in the cerebrospinal nerves, *A. Res. Nerv. & Ment. Dis. Proc.*, **15**:3-34, 1935.
 89. REIN, F. H. Zur Physiologie des Schmerzes, *Schmerz Narkose-Anaesth.*, **12**:129-39, 1939.
 90. ROSENTHAL, S. R. Histamine as possible chemical mediator for cutaneous pain; painful responses on intradermal injection of perfusates from stimulated human skin, *J. Appl. Physiol.*, **2**:348-54, 1949.
 91. ———. Histamine as possible chemical mediator for cutaneous pain. Dual pain response to histamine, *Proc. Soc. Exper. Biol. & Med.*, **74**:167-70, 1950.
 92. ROSENTHAL, S. R., and MINARD, D. Experiments on histamine as chemical medi-

- ator for cutaneous pain, *J. Exper. Med.*, **70**:415-25, 1939.
93. ROSENTHAL, S. R., and SONNENSCHN, R. R. Histamine as the possible mediator for cutaneous pain, *Am. J. Physiol.*, **155**:186-90, 1948.
 94. ROTHMAN, S. Beiträge zur Physiologie der Juckempfindung, *Arch. f. Dermat. u. Syph.*, **139**:227-34, 1922.
 95. ———. Über die Juckempfindung in ekzematöser Haut, *ibid.*, **150**:489-98, 1926.
 96. ———. Das Jucken und die juckenden Hautkrankheiten. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **14**:1:664-718. Berlin: J. Springer, 1930.
 97. ———. Physiology of itching, *Physiol. Rev.*, **21**:357-81, 1941.
 98. ———. Nature of itching, *A. Res. Nerv. & Ment. Dis. Proc.*, **23**:110-22, 1943.
 99. ———. Physiology and pharmacology of pruritus. In: EPSTEIN, S., Allergic pruritus. Its dermatologic management, pp. 1-8. St. Paul and Minneapolis: Bruce Publishing Co., 1952.
 100. ROTHMAN, S., and SHAPIRO, A. L. Pharmacodynamics of vehicles and drugs in dermatologic therapy, *M. Clin. North America*, **33**:263-79, 1949.
 101. ———. Itching. In: MCBRYDE, Signs and symptoms, pp. 713-37. 2d ed. Philadelphia: J. B. Lippincott Co., 1952.
 102. SACK, W. T. Über die psychogene Komponente des Pruritus und der pruriginösen Dermatosen, *München. med. Wchnschr.*, **69**:148-50, 1922.
 103. ———. Psyche und Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **4**:2:1302-82. Berlin: J. Springer, 1933.
 104. SANDERS, F. K. Special senses, cutaneous sensation, *Ann. Rev. Physiol.*, **9**:553-68, 1947.
 105. SCHNECK, J. H. Meralgia paresthetica: age incidence, *J. Nerv. & Ment. Dis.*, **105**:77-80, 1947 (literature).
 106. SCHUMACHER, G. A. The influence of inflammation on the pain threshold of the skin in man, *A. Res. Nerv. & Ment. Dis. Proc.*, **23**:166-72, 1943.
 107. SCHUMACHER, G. A.; GOODELL, H.; HARDY, J. D.; and WOLFF, H. G. Uniformity of the pain threshold in man, *Science*, **92**:110-12, 1940.
 108. SEITZ, P. F. D. Psychocutaneous aspects of persistent pruritus and excessive excoriation, *Arch. Dermat. & Syph.*, **64**:136-41, 1951.
 109. SHAPIRO, A. L., and ROTHMAN, S. Pruritus ani: a clinical study, *Gastroenterology*, **5**:155-68, 1945.
 110. SHELLEY, W. B., and MELTON, F. M. Relative effect of local anesthetics on experimental histamine pruritus in man, *J. Invest. Dermat.*, **15**:299-300, 1950.
 111. SHERRINGTON, C. S. The integrative action of the nervous system. New Haven: Yale University Press, 1947.
 112. SIGEL, H. High frequency square-wave current in dermatology: epilation, iontophoresis, cutaneous sensory threshold. Preliminary report, *J. Invest. Dermat.*, **15**:167-72, 1950.
 113. ———. Cutaneous sensory threshold stimulation with high frequency square-wave current. I. The relationship of electrode dimensions to the sensory threshold, *ibid.*, **18**:441-45, 1952.
 114. ———. Cutaneous sensory threshold stimulation with high frequency square-wave current. II. The relationship of body site and of skin diseases to the sensory threshold, *ibid.*, pp. 447-51.
 115. SINCLAIR, D. C. Observations on sensory paralysis produced by compression of human limbs, *J. Neurophysiol.*, **11**:75-92, 1948.
 116. SKOUBY, A. P. Sensitization of pain receptors by cholinergic substances, *Acta physiol. Scandinav.*, **24**:174-91, 1951.
 117. ———. The organization and function of peripheral receptors with special reference to the effects of acetylcholine and histamine, *Acta dermat.-venereol. (suppl. 29)*, **32**:332-42, 1952.
 118. SONNENSCHN, R. R.; CORNBLEET, T.; JANOWITZ, H.; and GROSSMAN, M. I. Inhibition of "itchy skin" with tetraethylammonium, *J. Invest. Dermat.*, **12**:321-23, 1949.
 119. SPERRY, R. W. Neurology and the mind-brain problem, *Am. Scientist*, **40**:291-312, 1952.
 120. STECKEL. Quoted by SACK (102).
 121. STETSON, R. H. The hair follicle and the sense of pressure, *Psychol. Monogr.*, **32**:1-17, 1923. Quoted by VON FREY (42).
 122. STOKES, J.; CHAPMAN, W. P.; and SMITH, L. H. Effects of hypoxia and hypercapnia on perception of thermal cutaneous pain, *J. Clin. Investigation*, **27**:299-304, 1948.
 123. STOKES, H. J.; KULCHAR, G. V.; and

- PILLSBURY, D. M. Effect on skin of emotional and nervous states; etiologic background of urticaria with special reference to psychoneurogenous factors, *Arch. Dermat. & Syph.*, **31**:470-99, 1935.
124. SULZBERGER, M. B. Dermatologic allergy. Springfield, Ill.: Charles C Thomas, 1940.
125. THÖLE. Über Jucken und Kitzeln in Beziehung zu Schmerzgefühl und Tastempfindung, *Neurol. Centralbl.*, **31**:610-17, 1912. Quoted by GRAHAM *et al.* (51).
126. TOWER, S. S. Unit for sensory reception in cornea, with notes on nerve impulses from sclera, iris, and lens, *J. Neurophysiol.*, **3**:486-500, 1940.
127. ———. Pain; definition and properties of unit for sensory perception, *A. Res. Nerv. & Ment. Dis. Proc.*, **23**:16-43, 1943.
128. UMRATH, K., and HELLAUER, H. F. Das Vorkommen der sensiblen Substanz und von Aktionssubstanzen abbauenden Fermenten, *Arch. f. d. ges. Physiol.*, **250**:737-46, 1948.
129. WALKER, A. E. Central representation of pain, *A. Res. Nerv. & Ment. Dis. Proc.*, **23**:63-85, 1943.
130. WALSH, F. M. R. Anatomy and physiology of cutaneous sensibility; critical review, *Brain*, **65**:48-112, 1942.
131. WEDDELL, G. The pattern of cutaneous innervation in relation to cutaneous sensibility, *J. Anat.*, **75**:346-67, 1941.
132. ———. The multiple innervation of sensory spots in the skin, *ibid.*, pp. 441-46.
133. ———. The clinical significance of the pattern of cutaneous innervation, *Proc. Roy. Soc. Med.*, **34**:776-78, 1941.
134. ———. Axonal regeneration on cutaneous nerve plexuses, *J. Anat.*, **77**:49-62, 1942.
135. ———. Anatomy of cutaneous sensibility, *Brit. M. Bull.*, **3**:167-72, 1945.
136. WEDDELL, G.; GUTTMANN, L.; and GUTMANN, E. The local extension of nerve fibers into denervated areas of skin, *J. Neurol. & Psychiat.*, **4**:206-25, 1941.
137. WEDDELL, G., and HARPMAN, J. A. The neurohistological basis for the sensation of pain provoked from deep fascia, tendon, and periosteum, *J. Neurol. & Psychiat.*, **3**:319-28, 1940.
138. WEDDELL, G.; SINCLAIR, D. C.; and FEINDEL, W. H. Significance of multiple innervation of cutaneous pain "spots" in relation to the quality of pain sensibility, *Nature*, **160**:27-28, 1947.
139. ———. Anatomical basis for alterations in quality of pain sensibility, *J. Neurophysiol.*, **11**:99-109, 1948.
140. WEITZ, J. Vibratory sensitivity as a function of skin temperature, *J. Exper. Psychol.*, **28**:21-36, 1941.
141. ———. Further study of the relation between skin temperature and cutaneous sensitivity, *ibid.*, **30**:426-31, 1942.
142. WHYTE, H. M. The effect of aspirin and morphine on heat pain, *Clin. Sc.*, **10**:333-45, 1941.
143. WINKLER, F. Studien über das Zustandekommen der Juckempfindung, *Arch. f. Dermat. u. Syph.*, **99**:273-334, 1910.
144. WOLF, S. G., and WOLFF, H. G. Pain. 2d ed. Springfield, Ill.: Charles C Thomas, 1951.
145. WOLFF, H. G., and GOODELL, H. The relation of attitude and suggestion to the perception of and reaction to pain, *A. Res. Nerv. & Ment. Dis. Proc.*, **23**:434-48, 1943.
146. WOLFF, H. G.; HARDY, J. D.; and GOODELL, H. Studies on pain: measurement of the effect of morphine, codeine and other opiates on the pain threshold and an analysis of their relation to the pain experience, *J. Clin. Investigation*, **19**:659-80, 1940.
147. ———. Measurement of effect on pain threshold of acetylsalicylic acid, acetanilid, acetophenetidin, aminopyrine, ethyl alcohol, trichlorethylene, barbiturate, quinine, ergotamine tartrate, and caffeine: analysis of their relation to pain experience, *ibid.*, **20**:63-80, 1941.
148. ———. Studies in pain: an analysis of the analgesic action of ethyl alcohol, *Tr. A. Am. Physicians*, **56**:317-20, 1941.
149. WOOLLARD, H. H. Observations on the terminations of cutaneous nerves, *Brain*, **58**:352-67, 1935.
150. ———. Intraepidermal nerve endings, *J. Anat.*, **71**:54-60, 1936.
151. ———. Continuity in nerve fibers, *ibid.*, pp. 480-91.
152. WOOLLARD, H. H.; WEDDELL, G.; and HARPMAN, J. A. Observations on the neurohistological basis of cutaneous pain, *J. Anat.*, **74**:413-40, 1940.
153. ZOTTERMAN, Y. Touch, pain and tickling: an electro-physiological investigation, *J. Physiol.*, **95**:1-28, 1939.

CHAPTER 6

Sweat Secretion

I. STRUCTURE OF ECCRINE AND APOCRINE GLANDS	154
II. INSENSIBLE ECCRINE SWEAT SECRETION AND ITS PERIODICITY	156
III. NUMBER, REGIONAL DISTRIBUTION, AND ACTIVITY OF ECCRINE GLANDS	158
IV. NONNERVOUS ECCRINE SWEATING	161
V. THE CHOLINERGIC NATURE OF NERVE FIBERS INNERVATING ECCRINE GLANDS	162
VI. THE PROBLEM OF ADRENERGIC SWEATING	166
VII. NERVOUS SWEATING	168
A. General	168
B. Axon-Reflex Sweating	168
C. Intrasympathetic Reflex Sweating	173
D. Generalized Reflex Sweating (Diencephalic and Cortical Reflex)	173
1. Efferent Pathways	173
2. Afferent Pathways	175
E. Thermal versus Sensory and Mental Stimuli	177
F. Disturbances of Sweat Secretion in Diseases of the Central Nervous System	178
G. Gustatory Sweating	178
VIII. APOCRINE-GLAND SECRETION	180
A. General	180
B. Influence of Age and Sex	182
C. Adequate Stimulation	183
D. Innervation	183
E. The Work of Shelley and Hurley	184
F. Composition of Apocrine Sweat and Its Odors	186
IX. SWEAT SECRETION AND BLOOD FLOW	188
X. THE MECHANISM OF ECCRINE SWEAT EXPULSION	189
XI. THE PROBLEM OF REABSORPTION	190
XII. HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS ON CHEMICAL CONSTITUENTS, STRUCTURE, AND FUNCTION OF SWEAT GLANDS	190

I. STRUCTURE OF ECCRINE AND APOCRINE GLANDS

THE physiology of the eccrine and apocrine glands will be discussed separately because of their different physiological functions. Eccrine glands, or the "small sweat glands," are distributed all over the surface of the body in man. They are tubular coil glands situated at the border of subdermis and dermis (Fig. 1). Embryologically, they

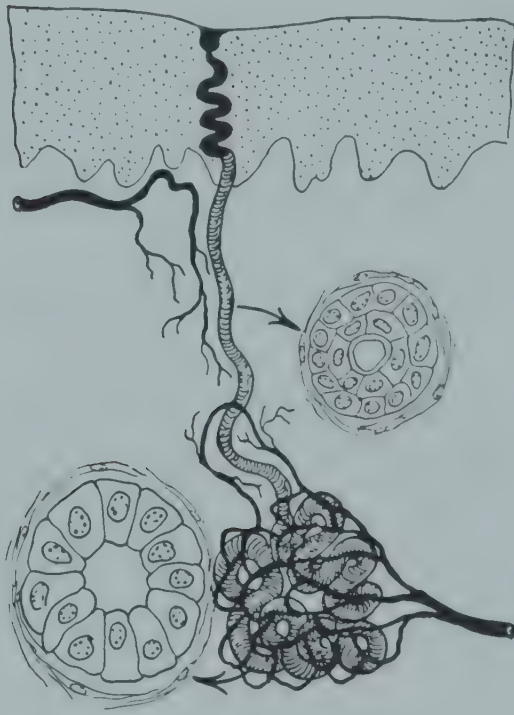


FIG. 1.—Coil and duct of sweat gland, with a cross-section of each and their arterial blood supply. From Lobitz and Mason (109). (Reproduced by permission of Dr. Lobitz and the American Medical Association.)

derive from the surface epithelium. As they extend downward and reach the subdermal fat, they coil because they cannot penetrate deeper (135). About half the coil consists of secretory epithelium, and half of it is duct. The two parts can be distinguished histologically. Both have two layers of epithelial cells, but the duct cells are about equal in size in both layers, while the secretory part has a luminal row of large light cells with

lightly stained nuclei (secretory cells) and a distal layer of flat cells with small, darkly stained nuclei (myoepithelia?). The duct ascends as a fairly straight tubule of equal diameter with no branches and no blind sacs (43). In the upper epidermal portion, however, it forms a spiraling coil reminiscent of tubular nephrons (178). Its opening on the surface is not related to the papillary and epidermal ridge systems (89) or to the openings of the hair follicles (135). There is no anastomosis between neighboring ducts (57). Each gland is a separate unit (Fig. 2).

The apocrine glands, or "large sweat glands," do not occur over the whole body surface in man. They are found in the axillae, perimammillary regions, mid-line of abdomen, mons pubis, perigenital, perineal, and perianal areas (54, 194), in the external ear canal (121), and in the nasal vestibule as appendages of the vibrissae (6). The anomalous occurrence of heterotopic apocrine glands was reported by Szodoray (180). Apocrine glands derive from the follicular epithelium of the hair (Fig. 3) and open into the follicular canal or follicular pore. They are larger than the eccrine glands, and their secretory coil is seated in the subdermis. The duct is wider and more tortuous than that of eccrine glands (Fig. 4). The microscopic structure of apocrine and eccrine glands is similar, but the muscular elements are more developed in the apocrine glands.

While eccrine glands are true secretory (merocrine) glands, producing the clear aqueous sweat responsible for heat regulation, the nature and function of apocrine glands is still under discussion. Phylogenetically, the apocrine glands are much older than the eccrine glands. The distribution of eccrine glands over the whole body surface is found only in the human race, while the apocrine system has become rudimentary in man (p. 180).

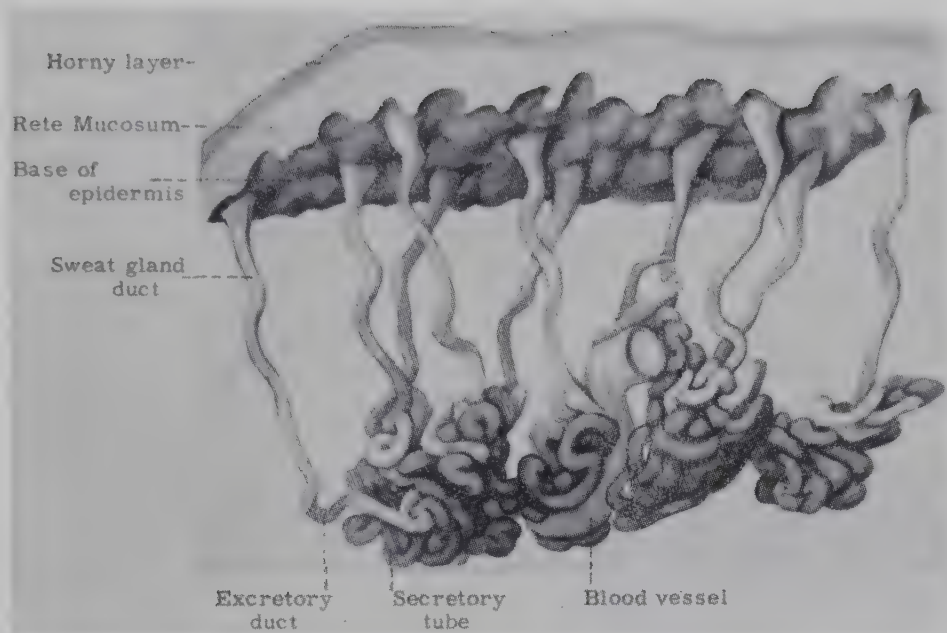


FIG. 2.—Eccrine glands. Each gland is a separate unit. From Pinkus (135). (Reproduced by permission of Springer-Verlag.)



FIG. 3. Apocrine gland budding from the upper portion of a developing hair follicle in the axilla of a 22-cm. fetus. From Pinkus (135). (Reproduced by permission of Springer-Verlag.)

II. INSENSIBLE ECCRINE SWEAT SECRETION AND ITS PERIODICITY

If the environmental temperature rises above a critical level, between 31° and 32° C. (or 88° and 90° F.), there is a more or less sudden outbreak of visible sweating over the whole body surface of man. A similar general sweat outbreak is observed if only

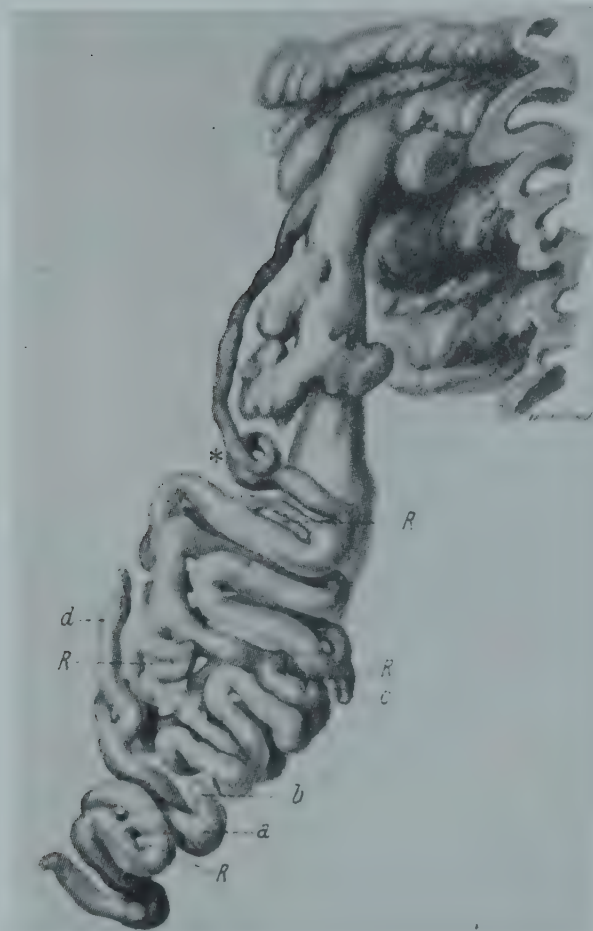


FIG. 4.—Apocrine gland. Drawing of a wax model. *R*, ring formation; *a*, sacculations; *b* and *c*, blind sacs; *d*, long blind sac; * beginning of duct. From G. Sperling, *Form der apokrinen Haardrüsen des Menschen*, *Ztschr. f. mikro-anat. Forsch.*, 38:241–52, 1935. (By permission of Akad. Verlag Geest & Portig K.G.)

parts of the body surface are heated. This is called “reflex sweating.”

At environmental temperatures below the critical temperature of reflex sweating, the sweat glands secrete microscopically visible sweat droplets. This grossly invisible func-

tion is periodic and involves alternating groups of sweat glands, as was first demonstrated by Jürgensen (71, 72, 73, 74) by direct microscopic observation. The droplets are invisible, not only because they are small, but also because as soon as they appear on the surface they immediately evaporate. This insensible sweat secretion constitutes one part of the total insensible water loss. The other part is loss of water through the epidermis, which is independent of glandular function. If the total insensible water loss is measured at average room temperatures in normal individuals before and after atropinization, it is found that atropinization decreases it by 10–30 per cent of its original value. Atropine blocks sweat-gland function but does not influence transepidermal water loss, as can be demonstrated in patients with multiple ectodermal dysplasia. The insensible water loss in these patients, who have no or only very few eccrine glands, is not influenced by atropinization (34) (see also chap. 9).

Observing single glands at comfortable temperatures over longer periods of time, Kuno's group (90) found the alternate functioning of glands confirmed. However, some glands were active more frequently than others, and some secreted sweat more continuously than others. In one case the observations were made on twenty-nine glands. Three were always active, twenty showed varied secretory responses, and six never responded.

Excitation of sweating by heat or exercise brings about primarily an increase in the number of functioning glands (90, 146, 147). As the intensity of the stimulus increases, the output of single glands will also increase (90, 146, 147). It appears that the output of single glands depends on the intensity of stimulation (90).

The periodicity in the function of single sweat glands was studied most frequently on the palmar surface of fingers but can be

demonstrated also on other parts of the body, e.g., dorsa of fingers and forearms (138). Kuno (90) found the periodicity of the palmar aspects of fingers to be more regular than that of the dorsal aspects; and he thought that the presence of "secretion beakers" (*Sekretbechers*) on the palmar surface might promote periodicity, whereas on the dorsal surface, where these orificial dilata-tions are not present, sweat flows away more rapidly and more irregularly. Periodicity at low temperatures was demonstrated by Takahara (181) after cannulation of single sweat glands on human palms. There are periods of a few minutes of inactivity, alternating with fairly uniform discharges, as demonstrated in Figure 5, in which the movements of the fluid meniscus in the capillary cannula are recorded. In response to repeated uniform stimulation of the same areas, the same glands do not always react first, though some are more frequently active than others. Between 20° and 27° C. atmospheric temperature, Randall (138) found no sweat gland on the human forearm which would secrete continuously for longer than 45 seconds. With increasing room temperature, the resting interval between secretory phases shortened, and a greater number of sweat glands functioned simultaneously. When the air was gradually heated to the critical temperature of general reflex sweating, the principal waves occurred at intervals of from 15 to 25 minutes. Randall (138) assumed that the periodicity of sweat secretion is caused by a periodicity of central nerve impulse discharges, and Kuno (90) noted that in reflex sweating the waves occur approximately simultaneously with vasomotor tonus waves. The relation of vasomotor impulses to periodic sweating in different regions was studied by Franke, Randall, Smith, and Hertzman (37).

With the modern method of infrared gas analysis, by which water-vapor concentration can be continuously recorded in an oxygen atmosphere, Albert and Palmes (131, 4, 5) came to the same results: at low sweating rates, there is a pulsatile rhythm of sweat secretion in the form of single bursts.

At moderate sweating rates, the average frequency is 6 to 7 bursts per minute. At high sweating rates, the frequency and amplitude of pulsations is damped. With simultaneous measurements, these authors have shown that the pulses are synchronous in different regions, and therefore they consider that the periodicity originates from a central stimulus. Periodicity of thermal sweating cannot be observed if heating is sudden and severe. Neither is there any

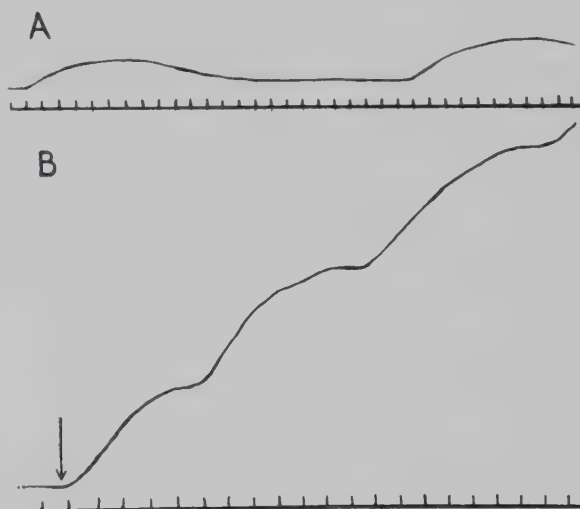


FIG. 5.—*A*, discharge of sweat from a single sweat gland of the palm (secretion at rest). Time marking indicates 5 seconds (Takahara). *B*, discharge of sweat from a single sweat gland of the palm during the grasping of a dynamometer. Time marking indicates 5 seconds (Takahara). Modified from Kuno. From Lobitz and Mason (109). (Reproduced by permission of Dr. Lobitz and the American Medical Association.)

periodicity when other sweat stimuli are applied intensely and continuously (90).

Lobitz and Mason (109) were the first observers to demonstrate that the composition of sweat is different according to whether the secretion is slow ("intermittent") or fast ("profuse"). They found that most constituents, such as chlorides, urea, uric acid, and ammonia, are much more concentrated in the sweat which is secreted intermittently than in profusely secreted sweat. They interpreted this finding by assuming that in intermittent slow secretion there is more opportunity for reabsorption of water from the ducts (p. 190). Like

Takahara (181), they observed sweat drop-lets rising and falling in the ducts when the sweat stimulus was weak and thought that this was due to a combination of secretion and smooth-muscle contraction (109). Randall and McClure (146, 139) estimated the average output of single sweat glands at

rest and in a warm environment to be 3.7-4.3 γ per minute on arms and legs, and 2-3 γ per minute on dorsa of hands and feet. On mild exercise, first the number of functioning glands increases, but later there is also an increased output of individual glands.

III. NUMBER, REGIONAL DISTRIBUTION, AND ACTIVITY OF ECCRINE GLANDS

The total number of sweat glands in a normal adult was estimated by Krause (85) to be 2.38 million. According to the research group of Kuno (90) and of Kawahata (77), they are certainly well over 2 million in number.

Per unit surface area, palms and soles have by far the most sweat glands, viz., about twice as many as forehead, forearms,

ity is not simply a function of the density of sweat glands but also depends on the size of the glands (196), on the output of individual glands, and on the responsiveness to different stimuli, which, in turn, depends on the conditioning of the nervous pathways.

In Ikeuchi and Kuno's account (61), the order in which sweat glands of different areas respond to thermoregulatory stimulation (at 39°-40° C. and 90 per cent humidity) is as follows: (1) strong and early response from forehead, neck, anterior and posterior aspects of trunk, dorsa of hands, and adjoining parts of forearms; (2) considerably less response from cheeks, lateral aspects of trunk, and greater parts of extremities; (3) little sweating on medial aspects of thighs, less in axillae, and least on palms and soles. Upper extremities sweat more than lower extremities, and whereas on the upper extremities sweating ability increases in the distal direction, the opposite is true for the lower extremities.

Kuno (90) speculated that this regional distribution may be purposeful. There is little thermal sweating where there is little chance for evaporation, e.g., medial aspects of thighs and axillae. More sweat might be produced on the head than on the extremities because the extremities have a larger surface as related to mass and therefore have more chance to lose heat by radiation and convection and are not so much in need of evaporative heat loss.

The regional distribution of sweating responses to sensory and mental stimuli differs greatly from that of thermal sweating. Palms and soles, responding late and weakly to heat stimuli, respond most promptly to

TABLE 1
DISTRIBUTION OF ECCRINE GLANDS
IN MAN (85)

(Figures Indicate Number of Sweat Glands per Square Inch of Surface Area)

Palms.....	2,736	Dorsa of feet...	924
Soles.....	2,685	Thigh and leg,	
Dorsa of hands.	1,490	medial aspect	576
Forehead.....	1,258	Thigh, lateral	
Chest and abdo-		aspect.....	554
men.....	1,136	Cheek	548
Forearm		Nape of neck...	417
Flexor aspect	1,123	Back and but-	
Extensor as-		tocks.....	417
pect.....	1,093		

or dorsa of hands, which rank next to palms and soles. Upper extremities have about twice as many as lower extremities, and there are twice as many sweat glands on the frontal aspect of the trunk as there are on the back. The old figures of Krause (85) (Table 1) on regional distribution have been confirmed by Japanese authors, with some additional data on racial differences. According to Yamada (201), the Japanese have relatively more sweat glands on the extremities than on the trunk, whereas this relation is reversed in Germans.

Physiologically, of course, sweating abil-

mental and sensory stimuli. The axillae take an intermediary position (61). Disregarding minor discrepancies, Kuno's findings on the distribution of thermal sweating ability were confirmed by subsequent investigators (196, 139, 51).

Weiner (196) found markedly different thermogenic sweat rates in different regions, the main feature being that head and trunk sweat more profusely than the extremities, with an irregular decrease in caudal and distal directions. Roughly, about 50 per cent of heat sweat comes from the trunk, 25 per cent from the lower limbs, and the remaining 25 per cent from the head and upper limbs. Weiner also compared the percentage which each region contributes to the total sweating with the percentage that the surface of the region contributes to the total surface area. In this way he arrived at a measure of "relative intensity" of sweating (see Table 2). The relative intensity of thermogenic sweating is greatest on the trunk and almost as great on the head. On hands and feet the relative intensity is only one-fifth to one-fourth that on the trunk, probably because of the poor response of palms and soles to thermal stimulation. Elsewhere on the extremities the relative intensity is one-third to one-half that of the trunk.

It is not known whether the regional differences are related to the density of the glands or to their different activity in different regions. Weiner (196) found that only the trunk participates regularly and intensely in the increase of sweating ability such as occurs in acclimatization to a hot environment (p. 265). It might be that there is a greater reserve of "unused" glands on the trunk and that this enables it to change more during acclimatization. Or perhaps the glands on the trunk are able to enlarge more than glands elsewhere, if acclimatization is connected with hypertrophy of sweat glands, which again is not known (196).

Randall (139) electrophoretically introduced mecholyl to stimulate sweat secretion maximally and counted the number of functioning sweat glands. In conformity with

histological counting, he found a greater number of functioning sweat glands on the dorsa of hands than on the forearms but, at the same time, a smaller sweat output on the backs of the hands. Again, in conformity with histological counts, he found a greater number of functioning glands on the chest than on the back and in the majority of cases considerably more on the forehead than on the cheeks. Randall was unable to elicit maximal response on the palms with his method. A much greater number of pores could be counted in the case of "spontaneous" (mental) sweating than after application of mecholyl.

TABLE 2

RELATIVE INTENSITY* OF SWEATING OVER VARIOUS REGIONS OF BODY SURFACE (196)

	Subject P.	Subject McK.	Subject W.
Head.....	1.4	1.4	1.0
Trunk.....	1.4	1.5	1.3
Thighs.....	0.8	0.5	0.8
Legs.....	0.9	0.7	1.2
Feet.....	0.7	0.4	0.2
Arms and forearms	0.6	0.8	0.7
Hands.....	0.2	0.2	0.3

* Relative intensity = Proportion of total sweat contributed by region ÷ proportion of total surface represented by region.

In a more recent contribution Randall, Hertzman, and Ederstrom (145) found thermal sweating to appear first on the calves and thighs, next on the forehead and lower trunk, and last on the upper extremities.

Older data have been essentially confirmed by Herrmann, Prose, and Sulzberger (51). In addition, these authors reported some new and important observations. They found a more staggered appearance of sweating in region after region, in most cases starting on the forehead, as contrasted with Kuno's (90) simultaneous onset of reflex sweating all over the body surface except palms and soles. In areas with lower sweating ability there was a longer latent period than in areas with high sweat delivery. There was a greater responsiveness to heat stimuli of the axillary glands than in

the experiments of Kuno (90). Most abundant sweat delivery was found in the mid-sternum at the level of the third costochondral junction, dermatologically a remarkable finding, in view of the yellow scaling spot in the very same area of patients with seborrheic dermatitis, in whom this change is a frequent stigma of their cutaneous constitution. The fact that the "mid-lines" on chest and back deliver more sweat than the corresponding lateral parts is clinically also important with regard to the mid-line distribution of seborrheic dermatitis. The authors pointed out the striking parallelism in the regional distribution

constant on repeated examination in the same subject. Weiner (196) comparing, thermal sweating of scalp and forehead, found in one subject an equal sweating ability of the two regions; in two other subjects, however, the scalp remained almost completely dry while the forehead sweated with unusual intensity.

The sweating ability of females is considerably less than that of males (p. 167). This sex difference was first demonstrated for sweating elicited by acetylcholine (76, 39, 64), but it is also true for sweating elicited by Prostigmin, pilocarpine, and heat (64, 51). Hardy, Milhorat, and Du Bois (49)

TABLE 3
COMPARISON OF TOTAL NUMBER OF SWEAT GLANDS OF PEOPLES LIVING
IN DIFFERENT CLIMATES

SUBJECTS	No. OF SUBJECTS	AGE	No. OF SWEAT GLANDS (THOUSANDS)		
			Min.	Max.	Mean
Ainu*	12	13-63	1,069	1,991	1,443 ± 52
Russians.	6	38-58	1,639	2,137	1,886 ± 59
Japanese, migrated to tropics..	4	14-35	1,839	2,603	2,168 ± 11
Japanese in Japan.....	11	6-35	1,781	2,756	2,282 ± 66
Filipinos.....	10	17-42	2,642	2,982	2,800 ± 23
Japanese, born in tropics.....	15	9-25	2,589	4,026	2,961 ± 61

* The Ainu is a Japanese aboriginal tribe living in the cold northern parts of Japan.

of thermogenic sweat and of lipids on the skin surface (p. 295). The quantitative variations of thermogenic sweat and lipid delivery paralleled each other surprisingly in these authors' experiments. Thus, in seborrheic dermatitis, anomalies of sweat secretion may play a role as well as anomalies of sebum secretion.

While it was previously taken for granted that sweat delivery is symmetrical, Herrmann *et al.* (51) discovered a natural asymmetry of sweat delivery in about 20 per cent of the subjects examined. Individual, general, and regional differences in sweating ability have been emphasized by all investigators. In Herrmann's experiments, sweat delivery on the temple varied tremendously (from 2.5 to 52.5 mg/2 sq cm/5 min) in different males, but was relatively

found that total vaporization from skin and lungs in males is greater than in females and that thermogenic sweating sets in earlier in males. These authors attributed this sex difference to the lower metabolic rate in females, which requires less evaporative heat loss. The newer findings, however, suggest that the sex difference is based on an inherently weaker response of sweat glands to acetylcholine in the female.

Interestingly, the sex difference was not apparent when the minimum effective concentration of acetylcholine was established by intradermal injection of exceedingly small volumes (0.01 cc.) (23). No sex difference was noted in sweating ability of palms and axillae in response to heat (51).

As to racial differences, there is little (39) or no (51) difference between the sweating

ability of the white and Negro races. However, recent findings of Kawahata and Sakamoto (77) indicate that the total number of sweat glands is smaller in people living in cold climates than in people living in the tropics (Table 3). These authors counted sweat glands by dividing the body surface into 20 areas, and taking three to five counts within each area in microscopic fields of 0.084 sq. cm., by the Jürgensen procedure (71). The phenomenon could be interpreted either as an adaptation phenomenon or as a true genetic racial difference.

Regional differences in the output of single glands are similar if sweat glands are stimulated by heat or cholinergic drugs. Some regional discrepancies were observed by Randall (139) and were confirmed by

Thurman and Ottenstein (130, 184). For instance, there is relatively more sweat on the forehead than in the axilla and on the trunk when sweating is elicited by mecholyl; but this is not the case in thermal sweating. Still it would be farfetched to conclude from such discrepancies that thermal (and emotional) sweating are not purely cholinergic (p. 163). One has to keep in mind the artificial nature of experiments done with local injection and/or electrophoresis of the cholinergic drugs. Quantitation of results is rather difficult with this method. Also one must remember that mecholyl is not the natural transmitter of sweat impulses. It differs from acetylcholine quite considerably in its greater resistance to the cholinesterases and in its slighter nicotinic action (28).

IV. NONNERVOUS ECCRINE SWEATING

Although, in everyday life, sweating is almost exclusively the result of nervous impulses, the sweat-gland cells are able to respond to direct application of heat, if this heat is sufficiently intense. This direct action is independent of nervous influence, can be elicited in denervated areas in its full intensity (158, 64), and is not abolished by atropinization or local anesthetics. It also appears after arrest of circulation (140, 7) and even if all soft tissues of one thigh had been divided and the nerves had degenerated (90). This local glandular response is in some respects analogous to the local response of sweat glands to acetylcholine. There is a close analogy also with erector muscles, which can be directly stimulated by cold, by electric current, or by epinephrine, independently of nervous influences, although the common physiologic stimuli of these muscles are adrenergic nerve impulses.

The nonnervous sweat response is strictly limited to the area of stimulation (158, 140), as contrasted with sweating due to nervous impulses, which always spreads beyond this area, because the impulses are carried farther by long or short reflex pathways. Saito (158) elicited direct stimulation of sweat

glands on the cat's paw by hot air (80° C.), which caused the temperature of the subdermis to rise to 40° C. In man he found the skin surface temperature to be between 43° and 46° C., when the local sweat reaction appeared. It was much more difficult to elicit it on palms and soles than elsewhere on the body. Randall (140) found the average critical temperature for local sweating in man to be between 39° and 45° C. skin surface temperature, somewhat lower than Saito's critical temperature but still considerably higher than the critical temperature for reflex sweating. In most cases local sweat appeared only when the surface temperature was well above 40° C. Randall (140), studying local sweating in response to heat, gives the following account: The latent period on direct gland stimulation is 1–8 minutes, mostly 1–2 minutes. The droplets are larger than those in reflex sweating; there is an excessive output of the individual glands. Not all glands of the heated area respond, but up to 70–80 per cent of them do. On cooling, sweating goes on for awhile, even after the skin temperature has fallen below the critical temperature. In contrast to reflex sweating, sweat due to direct heating is not subject to acclimatization or to

conditioning; there is no difference in the critical temperature in summer and winter. Previous treatment with atropine or procaine does not abolish the reaction completely but decreases it.

It was emphasized by Kuno (90), as well as by Randall (140), that direct sweat-gland stimulation by heat hardly ever occurs under natural circumstances. The heat required is so intense that it causes subsequent tissue damage, usually with edema and blister formation. Still it is conspicuous that if general reflex sweating is elicited by moderate local heating of a limited area, there is more sweat secretion at the site of direct heat exposure than there is elsewhere, at least initially (90, 138). Therefore, one may assume that if a gland receives some nervous impulse, the critical temperature for direct glandular excitation by heat is lowered. If this is true, the difference between amounts of sweat secreted at locally heated and reflex sweating areas should be greater—at least initially—the more intense the locally applied heat is. Whether this is so has not been explored. However, in this connection, a most important feature of local sweating was recently pointed out by Issekutz *et al.* (62). These authors experimented with local nonnervous sweating at two different levels of atmospheric temperature: at 18°–20° C. and at 29°–30° C. At the lower temperature level, difficulty was encountered in eliciting abundant local sweating by intense local heating. There was scanty sweating of 40–50 glands in an area supplied by 200–300 glands. But this scanty response always represented pure nonnervous sweating, which was not abolished or weakened by procainization. At the higher temperature level, local sweating was easier

to elicit by intense local heating, but such local sweating was rather easily weakened or abolished by procainization and, therefore, could not be regarded as a result of direct glandular excitation only. In other words, at atmospheric temperatures of 29°–30° C., it was hardly possible to elicit the pure phenomenon of local sweating, because local heating immediately also caused nervous sweating *in loco*, which was suppressed by procaine.

These observations show that it is not always possible to differentiate clearly between direct glandular activity and that induced by nervous stimulation. The fact that Randall (140) found a decrease in local sweating after application of procaine and/or atropine also indicates that he did not always deal with purely nonnervous glandular excitation, because procaine acts on nerve endings and atropine acts on the neuroglandular junction, but nothing is known about their action on secretory cells.

The best explanation that I can see is that intense local heating at high atmospheric temperatures induces a subthreshold general sweating impulse; the nervous impulse in itself is not sufficient to cause generalized sweating, but the increased nerve tonus, together with the local excitation of sweat-gland cells, elicits visible sweat secretion (p. 165). This assumption also explains why there is more sweating at the locally heated area when general reflex sweating sets in.

Theoretically, local nonnervous sweat may have a physiological role in protecting the skin from excessive rises in temperature through local increase of evaporative heat loss (7).

V. THE CHOLINERGIC NATURE OF NERVE FIBERS INNERVATING ECCRINE GLANDS

The innervation of eccrine glands, although anatomically sympathetic, is physiologically and pharmacologically “parasympathetic,” or, according to the nomenclature introduced by H. H. Dale (31), “cholinergic.”

The neurohumoral concept of nervous excitation postulates that, on stimulation of an autonomic nerve fiber, a chemical mediator, either nor-epinephrine or acetylcholine, is liberated at the nerve ending and acts

directly on the receptor organ. In contrast to all other postganglionic sympathetic fibers, which are adrenergic, sweat fibers liberate acetylcholine at their endings on stimulation, as directly demonstrated by Dale and Feldberg in 1934 (32). The effect of nerve impulses acting on eccrine glands thus becomes identical with the effect of acetylcholine on the same glands.

The concentration of acetylcholine liberated at sweat nerve endings can be extremely low and still effective. Coon and Rothman

ing agents, such as Banthine, Prantal, etc., do not hinder the liberation of acetylcholine following nerve stimulation but block the receptor site of the secretory cell so that it is unable to respond to acetylcholine.

In accordance with the neurohumoral concept, acetylcholine acts directly on sweat-gland cells, no matter whether the glands are innervated or not. Similarly, other choline esters and pilocarpine exert a direct action on the gland cells. There are numerous data to prove this thesis. How-



FIG. 6.—Histochemical demonstration of cholinesterase in eccrine glands and their cholinergic fibers. (Unpublished experiments of Dr. O. R. Aavik, Section of Dermatology, University of Chicago.)

(29) obtained positive sweat responses in man on intradermal injection by concentrations as low as $1:5 \times 10^{-7}$. The cholinergic response is greatly potentiated by physostigmine (eserine) and related compounds, which block cholinesterases—enzymes which split acetylcholine into choline and acetic acid and which are present in high concentration in and around cholinergic nerve endings (Fig. 6). If anticholinesterase drugs are administered, the naturally formed acetylcholine accumulates and has greatly intensified effects. This is the reason that physostigmine itself causes sweating.

Atropine and the newer cholinergic block-

ever, there is a paradoxical phenomenon which is hard to reconcile with this concept of direct action, namely, that sweat responses to locally injected acetylcholine and pilocarpine usually disappear completely soon after denervation of the gland. This is in contradistinction to the rule of Cannon (21), which states that smooth muscles and glands, innervated by cholinergic or adrenergic fibers, give abnormally strong responses to chemical stimuli after denervation ("sensitization phenomenon"), especially if the natural chemical mediator, nor-epinephrine or acetylcholine, is used as a stimulus. Weakened responses to pilocarpine after

denervation have been known for a long time, and it was repeatedly assumed that pilocarpine acts on nerve endings or on nerves rather than on gland cells (105). Kahn and Rothman (76) found that the local sweat response to intradermally injected acetylcholine quite regularly diminishes or disappears after postganglionic sympathectomies. However, they stated that the direct action of acetylcholine on sweat glands cannot be ruled out on the basis of such observations, because the sweat responses are sometimes positive, even though slight, for long periods after sympathectomies, during which time vari-

acetylcholine several hours after excision, when all nerve functions have ceased. Even *in vitro* the oxygen uptake by skin slices containing sweat-gland coils increases greatly on the addition of pilocarpine (127). In man, local responses to acetylcholine can be elicited in spite of complete paralysis of the peripheral sympathetic fibers by local anesthetics. On the other hand, in denervated areas the direct response is, in the majority of cases, abolished.

It appears that little attention has been paid to the phenomenon which may be labeled as "conditioning" of eccrine glands. This expression means that sweat glands re-

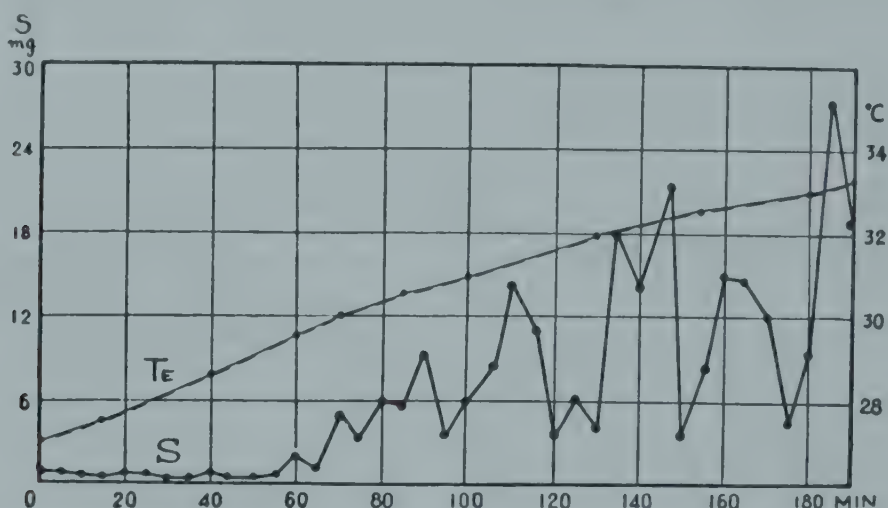


FIG. 7.—Moderate sweating due to a gradual rise in room temperature. *S*, perspiration on the chest; *T_e*, room temperature. From Kuno (90). (Reproduced by permission of J. & A. Churchill, Ltd.)

able responses are noted. Netsky (126) noted positive responses several years after denervation. A peculiarity of the weakening of the sweat responses in denervated areas is that it sets in very early, a few hours after denervation, and certainly long before the nerve has started to degenerate (76). The weakening of sweat responses to pilocarpine and acetylcholine is observed only if the postganglionic fiber has been separated from its ganglion cells, namely, in peripheral nerve injuries, in postganglionic sympathectomies, and in ganglionectomies. It is not observed after preganglionic sympathectomies (59, 23).

The problem remains unsolved. On the one hand, in the excised foot pad of cats, sweating can be elicited with pilocarpine or

act differently under different conditions in response to the same stimulus. One important conditioning factor is the environmental temperature. Response to acetylcholine is much more pronounced in a warm environment than in the cold (124, 29). The different conditioning in winter and summer is well known. Kuno (90) gives the following account: In winter, when sweating is induced by heat, either gradually or suddenly, there is always a latent period of about 30 minutes before visible sweat appears (Fig. 7). In constantly warm weather such latency is not present: as soon as the critical air temperature of visible sweat secretion is reached, sweat droplets appear. This is the "sensitive type" of sweating. Not only heat but any kind of sweat stimulus—drinking of

hot water, eating spicy food, faradization of the skin, or slight muscular exercise—is liable to cause sweating more promptly in summer than in winter (90). Kuno (90) also found that in cats, foot pads will fail to sweat on stimulation of the sciatic nerve if the foot is cooled to temperatures lower than 25° C. On the palms of man, mecholyl does not produce maximal response, except if there is a minimal subthreshold nervous tension (139).

In explanation of these observations it appears reasonable to assume a nerve tonus which does not represent an actual impulse with an effect which could be registered, but which is sufficient to increase the responsiveness of the gland to nonnervous stimuli. This tonus is higher in summer than in winter and practically zero when the skin is cooled. The tonus is lost by denervation, and response to local stimulation is weak or absent very soon after the gland has been denervated. The nerves have not yet degenerated, but they have lost their tonic effect as early as a few hours after severance. Observations on nonnervous sweating have led us to the same interpretation: The “nonnervous” local sweating is weakened by atropinization or procainization, because the local sweating is aided by nerve tonus if the environmental temperature is sufficiently high (p. 162).

It appears that, once the tonic effect is lost, acetylcholine and other cholinergic drugs are less likely to stimulate the glands than is direct application of heat. Two denervated cutaneous surfaces, when placed in contact with each other, will secrete sweat in a warm atmosphere (59). Also, denervated sweat glands not responding to acetylcholine will respond to locally applied excessive heat (64).

A remarkable (probably central) conditioning factor is alcohol intake. In Kuno’s (90) experiment the critical sudoriferous environmental temperature dropped from 32° to 27° C. the day after a drinking bout. This

could have been, however, also an indirect consequence of an increased tendency toward peripheral vasodilatation (p. 254).

The tendency toward excessive perspiration in hyperthyroidism, in hyperpituitarism, in convalescents after severe acute diseases, and in weakened individuals in general is not understood. It is certainly not due solely to increased metabolism or to any other caloric factor, because the sweat glands of such individuals show increased responsiveness also to sensory stimuli (90). Again the assumption of increased nerve tonicity in such cases would satisfactorily explain the situation.

According to this concept, commonly occurring hyperidrosis has two different mechanisms. One is an abnormal increase in nerve impulses, such as occurs in hyperidrosis caused by central nervous system lesions (p. 178) and in emotional hyperidrosis. The pathophysiological disturbance in these cases consists of increased nerve impulses, liberation of excessive amounts of acetylcholine, and subsequent excessive sweating. There is no increased responsiveness of the glands to acetylcholine demonstrable in emotional hyperidrosis (23). Another mechanism is an increased tonic effect of the sweat fibers, intensifying sweat responses to nervous and nonnervous stimuli of normal intensity. In such cases a certain concentration of liberated acetylcholine elicits a more intense sweat response than normal. Similarly, hypoidrosis and anidrosis can be due either to weakened nerve impulses, with decreased amounts of acetylcholine liberated, or to decreased responsiveness of sweat glands to acetylcholine because of low nerve tonicity, as is the case in postganglionic nerve damage. Decreased ability to sweat was demonstrated in such systemic diseases as pernicious anemia, secondary anemia, uremia, and late phases of diabetes (73). The mechanism of this hypoidrosis is unknown.

VI. THE PROBLEM OF ADRENERGIC SWEATING

In a number of mammals, such as the horse, sheep, cat, and dog, administration of epinephrine causes profuse sweating, which is not inhibited by atropinization (123). Neither is natural sweating following muscular exercise in the horse inhibited by atro-

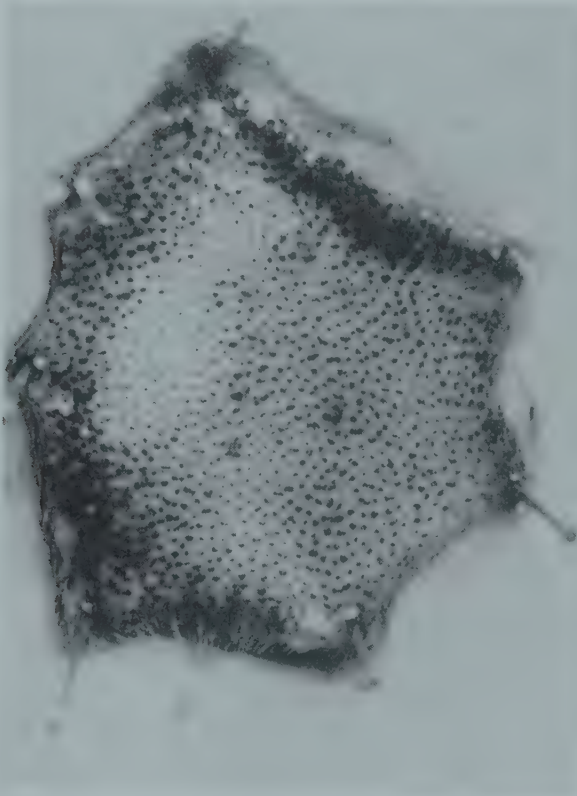


FIG. 8.—Sweating response to radiant heat in dog's skin removed from aspect of the thorax 5 minutes before heating. Sweating was rendered visible by the method of Wada. The black spots appear at the openings of hair follicles. Photographed after heating 10 minutes. From Aoki and Wada (7). (Reproduced by permission of Dr. Wada and the American Association for the Advancement of Science. Photograph courtesy of Dr. Wada.)

pine (123, 96). Because these animals, with the exception of the sheep, sweat also on administration of pilocarpine, which, like acetylcholine, stimulates cholinergically innervated structures, it was frequently assumed that sweat glands have a dual—adrenergic and cholinergic—innervation.

Recently, the dual response of sweat glands on the trunk of dogs was investigated

by Aoki and Wada (7). These glands respond equally to intradermally injected pilocarpine, acetylcholine, and epinephrine in normal as well as in denervated or excised skin. Sweating elicited by acetylcholine or pilocarpine is inhibited by atropine, that by epinephrine is not. These glands and the glands on the trunks of other domestic animals open into the follicular canal (25), as recently pointed out by Aoki and Wada (7). Their illustration (Fig. 8) shows clearly that the sweat droplets appear at the orifices of follicles. Thus these glands belong to the group of apocrine glands (p. 180). Functionally, they are certainly not comparable to human eccrine glands: they do not respond to nicotine or to excitement (struggling) and do not take part in systemic heat regulation (7). Their nerves cannot be stimulated to produce axon-reflex sweating (7).

Eccrine sweat glands in the cats' and dogs' paws are more comparable to human eccrine glands. A dual innervation was assumed for these glands, after it was observed that epinephrine on local injection stimulated them (90). Coon and Rothman (unpublished) occasionally saw minimal sweating on the cat's foot pad after intradermal injection of epinephrine, but this reaction was not reproducible regularly and was not comparable to the profuse and intense sweat flow following intradermal injection of acetylcholine. Much earlier, Langley and Uyeno (97) thought that epinephrine injected into the cat's foot pad has no more effect than the injection of saline solutions. Patton (134) was unable to block sweat-gland function in the cat's paw by Dibenamine, an adrenergic blocking agent, and came to the conclusion that sweat secretion in the cat's paw is a purely cholinergic function without any adrenergic component.

Recently, much attention has been paid to "adrenergic sweating" of eccrine glands in man (47, 48, 66, 172, 173, 12, 64, 188, 23). There can be little doubt that eccrine sweat droplets can be produced locally on the skin

surface with intradermal injections of epinephrine (Fig. 9) of as low a concentration as $1:10^{-8}$ (81). Adrenergic sweating appears within 2 minutes after injection and may last as long as 2 hours. Nor-epinephrine has a similar effect (48, 12). Other adrenergic compounds, such as Neosynephrine, can also act (47, 172, 173). Adrenergic blocking agents (ergotamine, Dibenamine, and Priscol) inhibit adrenergic sweating, and cholinergic blocking agents have no effect on it. In denervated areas adrenergic sweating could not be elicited (64).

Adrenergic sweating is not quite regularly reproducible. Haimovici (48) was able to elicit it in 84 per cent, and Sonnenschein *et al.* (66, 172, 173) in 76 per cent, of the subjects examined. The threshold of effective epinephrine concentration required was roughly in the same range as that of acetylcholine (23) but definitely higher than the threshold effective concentration of epinephrine on pilomotor muscles (28).

Systemic administration of epinephrine does not elicit sweating in man (48). Spontaneous sweating elicited by heat, exercise,

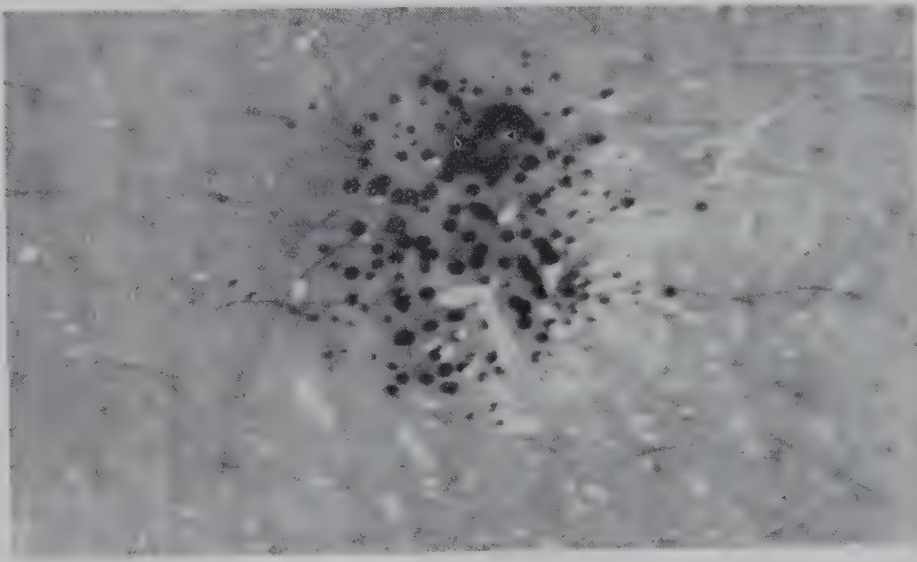


FIG. 9.—Sweat spots visualized by Wada's method at individual sweat pores of the extensor surface of the forearm, following intradermal injection of adrenaline of $1:10^7$. Photographed 18 minutes after injection. Not retouched. The white spots are caused by reflection of light. From Wada (188). (Reproduced by permission of Dr. Wada and the American Association for the Advancement of Science. Photograph courtesy of Dr. Wada.)

Haimovici (48) compared the effects of epinephrine, nor-epinephrine, and isuprel (*n*-isopropyl-nor-epinephrine), which was thought to be Cannon's inhibitory sympathin I (22). He found the greatest number of positive responses with epinephrine, less with nor-epinephrine, and only a small percentage of positive reactions with isuprel. It was thought that in the few cases when isuprel was effective it was hydrolyzed in the skin, yielding nor-epinephrine. Thus it appeared that the sudomotor effect was due to the excitatory component of the adrenergic mediator (sympathin E) and not to the inhibitory sympathin I.

and emotion can be completely blocked by local introduction of atropine and other cholinergic blocking agents and is not influenced by adrenergic blocking agents (134, 23). All this suggests either that adrenergic sweating is not a physiological phenomenon or, if the natural nervous sweating mechanism has an adrenergic component, that this plays an auxiliary role which is not essential.

To explain such an auxiliary role, one may assume that adrenergic agents contract the myoepithelia of the sweat glands. These structures have been described as being situated between the layers of epithelial cells

and the basement membrane, but as not occurring in all the sweat glands (90). Contraction of these muscular elements may express preformed sweat droplets, and thus adrenergic impulses may cause "sweating," without actually acting on the secretory cells.¹

Elicitation of sweating by locally applied epinephrine is followed by a refractory state of the glands in the stimulated area for 1–24 hours; this area will remain dry, in a state of general sweating (173). This observation, too, is compatible with the assumption that epinephrine acts on muscle cells rather than on secretory cells: a spastic state of the muscular coat which originally promoted expression of sweat may hinder expulsion later on. It seems that if epinephrine actually contracts the myoepithelium, this effect is independent of its vasoconstrictive action. In venous congestion, epinephrine has a minimal vasoconstrictor, but a maximal sudomotor, effect (173).

There is but one observation which is hard to reconcile with our assumption:

Haimovici (48) finds that Dibenamine, a compound blocking adrenergic impulses, considerably decreases not only epinephrine-induced, but also spontaneous, sweating of the palms, demonstrable by an increased electrical resistance. This finding reminds one, of course, of the very special and particular position of palmar and plantar sweat glands (p. 177). However, contrary results were reported by Chalmers and Keele (23); and Haimovici's finding is hard to reconcile with that of Sonnenschein (173), who tested twelve persons and was unable to elicit sweating on the palms by intradermal injection of epinephrine. Thus the physiological role of "adrenergic eccrine sweating" in man remains highly problematical.

Concerning apocrine glands in domestic animals, the available data strongly suggest that they have a dual—adrenergic and cholinergic—innervation. In man this problem is not settled. Shelley and Hurley (166, 168) insist that human apocrine glands are innervated solely by adrenergic fibers (p. 183).

VII. NERVOUS SWEATING

A. GENERAL

Sweating can be elicited from the periphery (1) by direct stimulation of sweat glands (p. 161) and (2) by nervous excitation. The nervous excitation is a reflex phenomenon and can be (a) axon reflex, (b) intrasympathetic reflex, (c) spinal or bulbar reflex, and (d) diencephalic or cortical reflex. Direct stimulation of sweat glands can be achieved by excessive heat (p. 161), by acetylcholine and other choline esters (p. 163), by pilocarpine, and by hexadienol (83). As stated above, if sweat glands are stimulated directly, sweating is restricted to the area stimulated, whereas in sweating due to nervous impulses the response is more widespread. In axon-reflex sweating, the size of the responding area depends on the number of axons, the ramifications of which have

been stimulated. In the intrasympathetic reflex the whole supply area of the excited ganglia will respond. Spinal-reflex sweating appears in a segmentary arrangement, and sweating is general in the diencephalic reflex.

Physiologically, the most common event is generalized sweating in response to either localized or generalized thermal stimuli. The efferent path of this long reflex is well established, but the afferent path is still controversial.

B. AXON-REFLEX SWEATING

In 1938, Bickford (17) and Wilkins, Newman, and Doupe (198) described a widespread sweat response to punctiform faradic stimulation of the skin and interpreted this response as an axon reflex. In 1939, Coon and Rothman (27, 156, 29) reported that the same widespread response can be elicited by acetylcholine and by other drugs with nicotine-like action.

1. Wada *et al.* (189) believed that potassium salts injected intradermally caused sweating by stimulating myoepithelial cells.

On intradermal injection of acetylcholine (29) or of mecholyl (124) (acetyl- β -methylcholine) solutions and other choline esters, the sweat response appears on top of the injection wheal within 5–10 seconds and slowly increases in intensity and extent, corresponding to the slow diffusion of the drug. In my experience, in the majority of cases the maximum spread, even with high (very unphysiologic) concentrations ($1:10^4$ – $1:10^3$), is only a few millimeters away from the wheal and is not reached until 5–10 minutes after injection, if the volume does not exceed 0.1 cc. Randall *et al.* (143) found patterns which suggested the participation of cutaneous lymph channels in the convection of the drug away from the site of the injection. The intensity of this reaction decreases with decreasing concentration of the drug.

This sweat response to acetylcholine is analogous to the response of other structures with autonomic cholinergic innervation, such as smooth muscles of bowels, salivary glands, etc. They are all stimulated by acetylcholine in the same manner as if parasympathetic nerve impulses stimulated them. The response is blocked by atropine and other cholinergic blocking agents, because these agents block those receptor groups of the cells which otherwise respond to acetylcholine. Infiltration of the skin by local anesthetics does not abolish the response—an indication that the action is directly on the sweat-gland cells and not on nerve endings or nerve fibers, which are blocked by infiltration anesthesia. The direct response can be obtained with any concentration of acetylcholine solutions from $1:50,000,000$ upward.

In addition, intradermal injections of acetylcholine in concentrations of from $1:10,000$ to $1:80,000$ produce still another type of sweat response, distinct and separate from the one described above (27, 156, 29). This is a sudden widespread outbreak of sweat droplets around the injection wheal in an area about 5 cm. in diameter, with occasional isolated groups of secreting pores outside the main responding area. This re-

sponse appears immediately, is fully developed within 1–2 minutes after injection, and can easily be distinguished from the slowly spreading sweating caused by direct action on gland cells. In contrast to the latter, the widespread reaction is abolished by preceding infiltration anesthesia.

The action of acetylcholine on cells and structures with cholinergic innervation is referred to as “muscarinic action,” because this action is similar to that of muscarine, a poisonous alkaloid occurring in mushrooms. This action is inhibited by atropinization and is greatly enhanced by physostigmine and other anticholinesterases (p. 163).

The widespread sweat reaction is due to a second pharmacological effect of acetylcholine, referred to as “nicotinic action” (40), because it is similar to the action of nicotine. Nicotinic actions of acetylcholine, in general, have three special features: (1) they are not abolished by atropine; (2) they are enhanced by physostigmine to only a slight degree; and (3) the stimulating nicotinic action of acetylcholine changes into a paralyzing one when the concentration is increased. For instance, low concentrations of acetylcholine stimulate sympathetic ganglion cells, but high concentrations paralyze them.²

The widespread sweat reaction can be elicited with nicotine itself (Fig. 10) and with other drugs having nicotinic action, as well as with acetylcholine. While acetylcholine has a dual effect—muscarinic action on gland cells, nicotinic action on nerves—nicotine elicits only the sudden widespread reaction.

Evidence has been presented (27, 156, 29) that the reaction set up by drugs with nicotinic action is due to an axon-reflex impulse (p. 170). The reaction is abolished by preceding infiltration anesthesia; it is not impaired by block anesthesia or by fresh

2. This latter feature of the nicotinic action cannot (64) be demonstrated in axon-reflex sweating if elicited by acetylcholine and never was claimed to be demonstrable. It is easily shown, however, if the axon reflex is elicited by nicotine or α -lobeline (27, 156).

nerve section; it can be elicited in the freshly extirpated toe pad of the cat; but it is abolished when the postganglionic neurons have degenerated.

The cholinergic axon-reflex sweating is in all respects analogous to the adrenergic pilomotor axon reflex (101, 191, 155, 28, 156)



FIG. 10.—Sweat response to intradermal injection of 1:100,000 nicotine sulfate visualized by Minor's method. From Coon and Rothman (29). (Reproduced by permission of the Williams & Wilkins Company.)

(Fig. 11). In both cases the effect is the result of an impulse involving the terminal ramifications of sympathetic fibers. Some rami are hit by the stimulus, and the impulse runs in a centripetal direction until it reaches the highest point of ramification. Then it spreads centrifugally over all the rami of the axon. It is for this reason that this pathway (Fig. 12) is not eliminated by nerve block or by fresh nerve cut above the highest point of ramification. By histologi-

cally controlled cuts through the skin and by observing the spread of the reflex impulse, it could be shown that the highest point of ramification is situated in the lower half of the corium (28). When the section of the nerve has caused degeneration of the axon, with its peripheral ramification, in about 2 weeks, the sympathetic axon reflexes can no longer be elicited in the area supplied by the degenerated nerve, because the ganglion cell is the nutrient center of the axon and some time after separation from this center the peripheral axon rami will die.

The pilomotor response involves adrenergic, and the sudomotor response involves cholinergic, fibers. They can be separated from each other either by eliminating the pilomotor response with adrenergic blocking agents—for instance, ergotamine tartrate—or by eliminating the sudomotor response with cholinergic blocking agents—for instance, atropine. The nicotinic impulse is identical in both instances; but after the impulse has run centrifugally, it liberates norepinephrine at pilomotor fiber endings and acetylcholine at sweat fiber endings. The blocking agents do not block the nerve impulse but the action of the liberated chemical mediators on the target organs.

Posterior-root ("sensory") fibers are not involved in these sympathetic local reflexes. The sympathetic fibers in which the axon reflex occurs are 10–20 times as sensitive to local anesthetics as are posterior-root fibers. Therefore, with low concentrations of procaine, the sympathetic reflexes can be abolished without altering the red-flare axon reflex of posterior-root fibers in response to histamine (29) (p. 93).

In the special case of eliciting the sudomotor axon reflex by acetylcholine, the reaction is initiated by the nicotinic action; but, strangely enough, the resulting widespread sweating is caused by the muscarinic action of the endogenously liberated acetylcholine. This makes understandable the anomalous situation that, although nicotinic actions in general are not inhibited by atropine, the sweat axon reflex cannot be elicited after atropinization, because atropine acts

on the neuroglandular junction in the efferent limbs of the reflex. It is not surprising to find that axon-reflex sweating is weaker in

confirm the results of Grossman and his associates (42, 63) under a great number of variable experimental conditions. Further

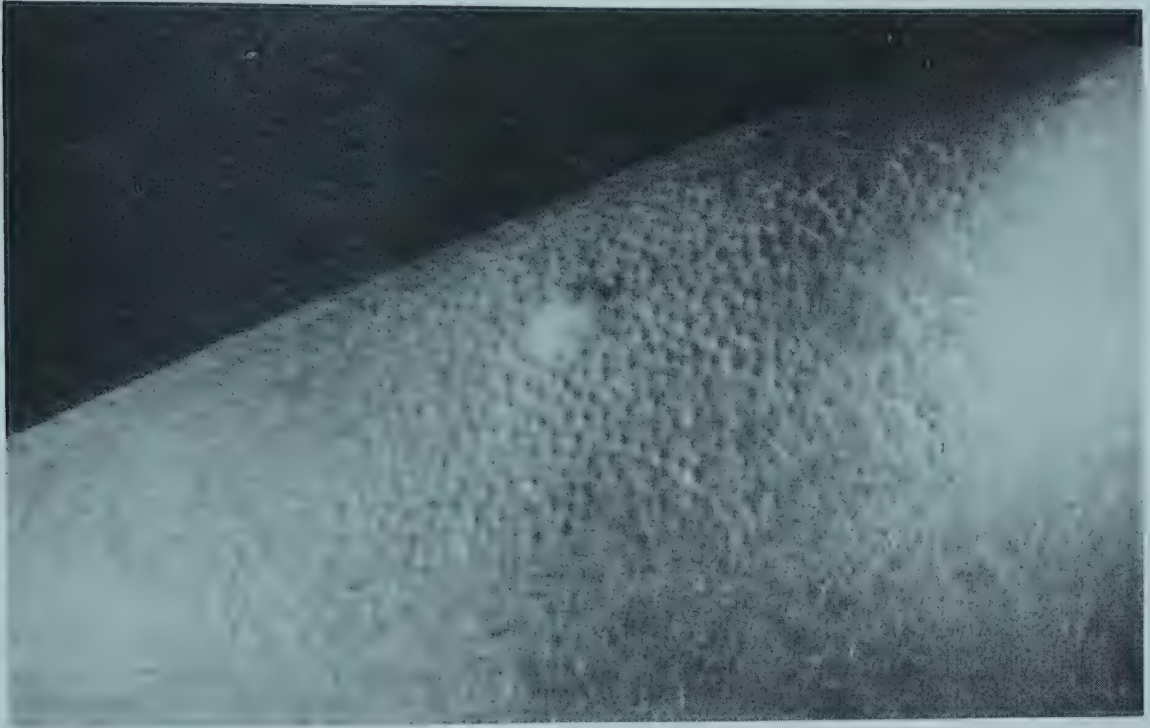


FIG. 11.—Pilomotor axon-reflex response to an intradermal injection of a 1:100,000 solution of nicotine sulfate. From Rothman and Coon (155). (Reproduced by permission of the American Medical Association.)

women than in men (64), because the sweat glands of women show a diminished response to the muscarinic action of acetylcholine (p. 160). The difference certainly does not prove a sex difference in nerve conduction.

The highest points of ramification of the arborizing sweat fiber axons behave like autonomic ganglion cells in so far as they are stimulated by small concentrations, and are paralyzed by large concentrations, of nicotine. This similarity has been further emphasized by the finding that the axon reflex can be blocked by tetraethylammonium (63), a drug which is known to block ganglionic synapses but not nerve fibers. The blocking of the sweat (and pilomotor) axon reflex was observed when acetylcholine was injected 1–2 minutes after infiltration of the skin with 1 per cent tetraethylammonium solution. Although some negative results also have been reported (62), Lorincz (111) in the author's laboratory was able to

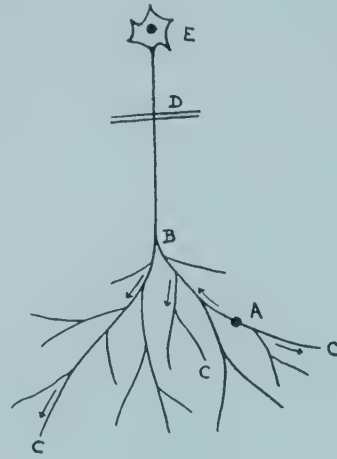


FIG. 12.—Scheme of the axon reflex. *A*, point of reception; *B*, highest point of ramification; *C*, nerve endings; *E*, ganglion cell. From Rothman and Coon (156). (Reproduced by permission of the American Medical Association.)

confirmation has come recently from Wada *et al.* (189). These authors have further stressed the similarity of the ramification

points to ganglia by demonstrating similar behavior to calcium, magnesium, and potassium salts and to adrenaline and by pointing out that the sensitivities to acetylcholine and nicotine are of approximately the same order in the axon reflex and in ganglia.

In spite of the pharmacological similarity of the "skin-nerve units" to ganglion cells and in spite of their apparent autonomy, it must be kept in mind that these units are not so independent as the whole neuron is. They function for a while after their connection with the nutrient ganglion cell is severed, but, like any axon, they degenerate within 2 weeks after the severance of the connection.

In a recent publication Wada *et al.* (189) confirmed the previous work by Coon and Rothman (27, 156) on axon-reflex sweating in all details and added some further data. They were able to elicit axon-reflex sweating by intradermal injection of hypertonic sodium salts; but they stated that sodium salts must have a somewhat different mode of action on the receptor end of the axon, because the reflex produced by sodium salts is not inhibited by tetraethylammonium. Whereas the ganglion-blocking effect of tetraethylammonium may be based on competition with the material eliciting the impulse (133), the effect of sodium chloride is probably the result of a sudden disturbance of ionic balance at the receptor points, and tetraethylammonium is unable to compete with this effect. Potassium salts do inhibit the axon reflex elicited by sodium salts.

By means of a "double-band method," inhibiting the spread of the acetylcholine injected but not inhibiting the spread of the nervous impulse, Wada *et al.* (189) attempted to distinguish the point of attack of different pharmacological agents, i.e., to establish whether they act on the receptor or the effector points of the axon. They found that, with the exception of atropine, which acts on the neuroglandular junction, all inhibitory effects so far studied, namely, those of procaine, calcium, magnesium and potassium salts, tetraethylammonium, and adrenaline, are on the receptor and not on the

effector points as long as the concentrations are not too high. The apparently different sensitivities of the receptor and effector points of the axon led the authors to the tentative conclusion that these points are possibly not identical. But their results can be well interpreted also by assuming that the same points act differently according to whether they *function* as receptor or effector points.

The concentrations of acetylcholine required to elicit axon-reflex sweating are rather unphysiologically high.³ Still, it is possible that such concentrations are liberated in response to severe injury. The first pertinent finding was the observation of the sweat axon reflex in response to strong faradic stimulation of the skin (17, 198). This reaction might have come about either by liberation of unphysiologic amounts of acetylcholine in the skin or by direct stimulation of nerves without chemical mediation. In any case, this observation indicates that in response to morbid stimuli the sudomotor axon reflex may very well occur in disease. Rothman and Coon (156) stated that external mechanical, chemical, thermal, and electrical stimuli may act on the point of nervous reception, from which axon reflexes have been elicited experimentally, either directly or mediated by acetylcholine liberation. Shelley *et al.* (167) pointed out that localized hyperhidrosis, which is seen at the periphery of inflammatory skin lesions (p. 178), may well be the result of axon reflexes.

The "nicotine test" (intradermal injection of $1:10^5$ nicotine sulfate solution and observation of the pilomotor and/or sweat axon reflex) can be used for examining the integrity of the peripheral mixed nerve in the same way as histamine is used. Whereas absence of the histamine flare indicates degeneration of peripheral sensory neurons, absence of the pilomotor and sweat reflex indicates degeneration of peripheral sym-

3. Wada *et al.* (189) have theorized that, even in physiological sweating, the acetylcholine liberated may stimulate receptor points of the axon reflex. Because of the quantitative situation, this is not probable.

pathetic neurons. I carried out nicotine tests in surgical grafts and was able to follow with this method the growing-in of pilomotor fibers into the new graft (86). H. L. Arnold used the reflex for examining nerve involvement in patients with tuberculous leprosy (8, 9), by using acetylcholine and metacholine, and found it in some respects superior to the histamine test.⁴

So far, experimentation to dampen sympathetic axon reflexes by large paralyzing doses of α -lobelin (29) or by the newer "antinicotinic" drugs with curare-like action (111) have been unsuccessful. Clinical utilization of tetraethylammonium and similar ganglion-blocking bases has not yet been tried.

C. INTRASYPHATHETIC REFLEX SWEATING

That an intrasympathetic sweat reflex exists was demonstrated by H. G. Schwartz (162) in 1934. After severing the central connections in the cat and dog, he removed all branches of the stellate ganglion except one gray ramus; the posterior roots also were severed. Under such conditions he obtained, on adequate peripheral stimulation, decreased skin resistance on the forepaw, a sign of sweat-gland stimulation. This effect was elicited by pressure, and therefore Schwartz assumed that the impulse originated in blood vessel walls. The afferent fiber carried the impulse through the gray ramus to its cell of origin in the stellate ganglion. There a synaptic relay transferred the impulse to the efferent neuron (Fig. 13).

Whether such a reflex can be elicited from more superficial structures is not known. Also, whether the afferent and efferent axons are actually different structures or whether, as in the axon reflex, the impulse runs centripetally and centrifugally in the same pathways remains to be investigated.

4. See also Arnold's recent report at the Xth International Congress of Dermatology (H. L. Arnold, Jr., The pilomotor response to intradermally injected nicotine: an aid in excluding the diagnosis of leprosy, *Excerpta med.*, Sec. XIII, 6:327, 1952).

In any case, the existence of such an intrasympathetic reflex seems to be highly significant for the physiology of sweating.

D. GENERALIZED REFLEX SWEATING (DIENCEPHALIC AND CORTICAL REFLEX)

1. Efferent Pathways

According to Guttmann and List (46), the efferent pathway consists of five neurons extending (1) from cortex to hypothalamus, (2) from hypothalamus to the medulla, (3)

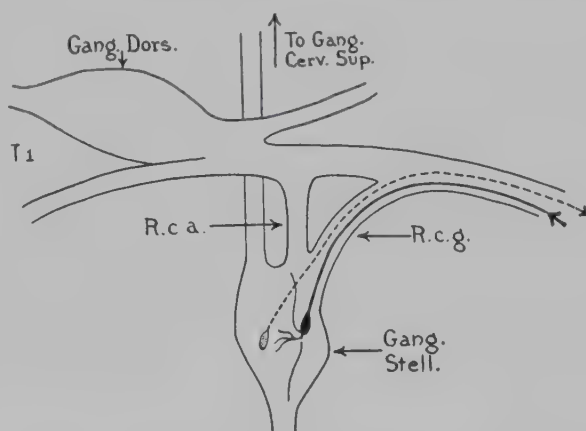


FIG. 13.—Scheme of mechanism responsible for the intrasympathetic reflex, showing an afferent fiber with its cell body in the stellate ganglion. *R.c.g.*, ramus communicans griseus; *R.c.a.*, ramus communicans albus. From Schwartz (162). (Reproduced by permission of the author and the American Physiological Society.)

from the medulla, partly crossed, partly uncrossed, to the lateral horn of the spinal cord, (4) from the lateral horn to sympathetic ganglia, and (5) from sympathetic ganglia to sweat glands. According to these authors (46), the main adequate stimuli are psychic for the first neuron, thermal for the second, gustatory for the third, and non-thermal skin stimuli for the fourth and fifth. Actually, the number of neurons and pathways between cortex and spinal cord are far from being clearly established (113, 107). However, the scheme of Guttmann and List indicates the pathways of efferent impulses in broad lines.

Sweating, like other autonomic functions, has a cortical representation in the premotor

area, also called "area 6 of Brodmann" or "area frontalis agranularis" (113). When this area of the cortex is destroyed in the cat, no sweating can be induced on the contralateral side by "emotional" stimuli, such as a loud whistle or tapping on the nose (163). The cortical representation accounts in man for the purely "mental" sweating. Stimulation of the cortex by sensory stimuli acting on the conscious psychic sphere elicits sweating primarily on palms and soles but eventually all over the body surface.

However, the cortical connections of the sweat pathways are by no means necessary to elicit generalized reflex sweating. The subcortical centers suffice to perform this task if the sensory stimuli are sufficiently intense. In Schwartz's experiments (163), cats with destroyed premotor area, although not responding to purely frightening sensory stimuli, did respond with sweating to strong pressure of the forelimbs or to pinching of the tail. Thus no sharp border line can be drawn between mental and nonmental sweating, as it is impossible clearly to separate conscious and subconscious events in general. This becomes particularly evident from the fact that in the so-called "premotor syndrome" of man, in which only emotional sweating should be disturbed, lack of sweating on the contralateral side was observed also in response to environmental heat (80, 79).

The subcortical centers of sweating are situated in the hypothalamus, which acts as a thermostat, regulating body temperature. In a long series of classical physiological experiments Ranson and his associates (150, 114, 24) dealt with this function of the hypothalamus and with the exact localization of the centers. With the hypothalamus intact, the homeostatic thermoregulation of mammals is well maintained, in spite of destruction of higher centers (p. 244). Wang and Richter (193) dealt with the possible role of the tuber cinereum as a subcortical sweat center.

Hypothalamic efferent sweat impulses descend in the brain stem, both crossed and

uncrossed (107). Disturbances of sweat secretion in lesions of the pons indicate pathways from the diencephalon to the cord via the pons, with crossings in its oral parts (107). Frontopontile and temporopontile tracts were found to be involved also in physiological experiments (98). Some of the crossing seems to occur below the stem in the spinal tracts (14) (Fig. 14). In the medulla most fibers travel through the lateral reticulospinal tract (107). Some evidence was presented for the participation of pyramidal tracts in the efferent pathways of sweat impulses (78). In the cord new neurons start in the lateral horn (see Fig. 14). They form the myelinated preganglionic fibers of the descending sweat pathways and descend in the lateral and anterolateral columns (14). They leave the cord through the anterior roots, pass through the white rami communicantes, and end up synapsing within the sympathetic ganglia. From the point of view of cutaneous reactions it is remarkable that it was possible to separate experimentally the pathways of sweat and pilomotor impulses in the mid-brain and in the cord (14, 15). Physiologically, too, the impulses in the sympathetic pathways, including the sympathetic vasoconstrictor impulse, are independent of one another. Clinically, although they often appear synchronously, they can well be separate. Whereas thermoregulatory sweat is not connected with vasoconstriction or pilomotion, such an association can be observed in "cold sweat." The simple fact that there are "warm" sweat and "cold" sweat (sweat with and without cutaneous hyperemia) sufficiently indicates the mutual independence of sympathetic impulses to the skin. The postganglionic nonmyelinated fibers originate in the ganglion cells of the sympathetic chain. They pass through the gray rami communicantes to join the peripheral mixed nerve and end with multiple ramifications around the sweat-gland coils.

On direct stimulation of any part of this efferent pathway, acetylcholine is liberated at the sweat fiber endings, which, in turn, cause secretion of sweat (32).

There has been much discussion about the existence of parasympathetic cranial and spinal cholinergic sweat fibers, in addition to sympathetic fibers, because of sweating observed after interruption of sympathetic pathways (93). List and Peet (105) observed persistence of sweating on the head after interruption of all sympathetic fibers supplying the head from the superior cervical ganglion. They saw complete anidrosis only after denervation of branches of the trigeminal nerve. However, these authors considered, with good reason, the fact that the head is richly supplied with other (non-sudomotor) cholinergic fibers, and thought it possible that acetylcholine, liberated at the endings of these fibers, may diffuse to sweat glands and stimulate them in an indirect way. The cholinergic fibers in question are probably identical with the efferent vasodilator posterior-root fibers (105). Altogether, nonsympathetic innervation of sweat glands (parasympathetic fibers passing the anterior roots, as suggested by Kuré [93]) has not been proved conclusively.

From neurological experiments in man, Foerster (36) concluded that dorsal roots carry fibers which inhibit sweating. Such inhibition by "sensory fibers" was not found, however, in animals either by Langley (95) or by Schilf and Mandur (161).

2. Afferent Pathways

In thermoregulatory sweating, general sweat outbreak is effected in two ways: (1) warmed blood, arriving from the periphery to the brain, stimulates hypothalamic thermoregulatory centers, and (2) afferent nerve impulses, arriving from the periphery, stimulate the same centers via a long reflex mechanism.

The operation of the first mechanism has been proved beyond doubt many times. However, it is often questioned whether the second mechanism actually operates. Originally, Filehne (35) thought that cutaneous warmth receptors initiate a sweating reflex via the central thermoregulatory apparatus, because he observed that thermoregulatory sweating is abolished by placing the hands

in cold water, although the body temperature is not changed by this procedure. Filehne thought that this observation proved the nervous mechanism of generalized sweating and of its inhibition. Contrariwise, Hill (53) maintained that even if

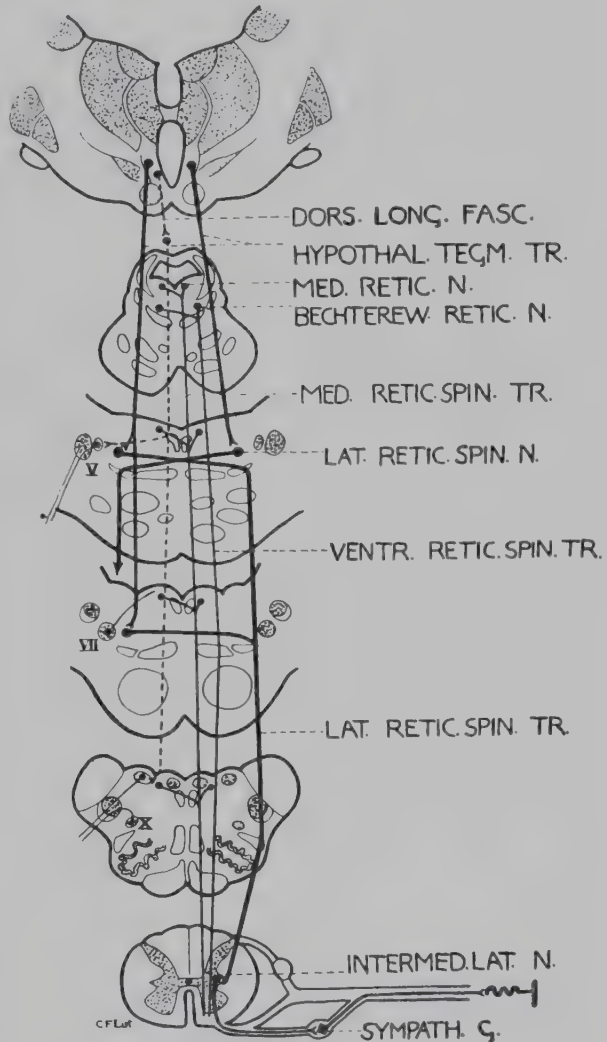


FIG. 14.—Diagram to show the probable pathways of thermoregulatory sweating (hypothalamo-tegmental and reticulospinal connections). From List and Peet (107). (Reproduced by permission of Dr. List and the American Medical Association.)

the rectal temperature is not changed by cooling the hands, the blood is being cooled, and therefore afferent nervous impulses need not be assumed. Adolph, too (3), thought "reflex" sweating does not come from a nervous reflex but exclusively from an increase in the heat content of the body.

Careful experiments carried out in Kuno's (90) laboratory have left little doubt that long reflexes eliciting general sweat outbreak do exist. In contrast to Hill, Kuno's associates found that even if the cooled limb is cuffed so that cooled blood cannot reach the center, there is inhibition of sweat outbreak by cooling an extremity. Kuno also showed that sweating may occur even with rectal temperatures falling and without any increase in blood temperature. Randall *et al.* (141, 142) showed that if one limb is heated and its circulation occluded, reflex sweating still takes place.

That this long reflex originates in the warmth receptors of the skin has lately been questioned by Issekutz *et al.* (62). These authors first experimented with capsaicine (an irritant rubefacient extract from *Capsicum annuum*), which on local application decreases the threshold of warmth perception. When an area treated with capsaicine is heated, there is an unusually intense warmth sensation. Nevertheless, such treatment does not facilitate reflex sweating in any respect. Second, the authors, applying cocaine by iontophoresis, created a situation in which there was complete analgesia and anesthesia but in which perception of warmth was not abolished. Such a condition can be produced easily, because the heat receptors are deep-seated and the myelinated fibers of the thermoreceptor system are relatively insensitive to local anesthetics. If such areas were heated, no reflex sweating could be elicited. The conclusion was that thermoreceptors do not participate in eliciting long reflex sweating. This conclusion is supported by the observation that very gradual heating, which does not cause heat sensation because of adaptation (p. 130), is fully effective in eliciting general sweating (90).

Therefore, other than thermoreceptor sensory pathways of reflex sweating must be considered. The fact that these pathways are easily blocked by local anesthetics in the skin indicates that probably thin, non-myelinated fibers carry the impulse from the skin centrally. There are two such sets of

fibers in the skin: the thin, pain-perceiving C fibers of Gasser and Erlanger (33) and sympathetic fibers. Pain-perceiving fibers can be ruled out on the basis that reflex sweating is not connected with pain sensation. Also, Issekutz *et al.* (62) found that in partial cocainization, with the pain sense still not paralyzed, no reflex sweating could be elicited from the cocainized areas. This observation points to the possible role of sympathetic fibers.

There are two experimental observations suggesting that the centripetal limb of the reflex arc in reflex sweating is constituted of sympathetic fibers, possibly the same fibers which also carry the impulse centrifugally. Schwartz (162) in 1934 demonstrated a special type of reflex sweating which took place entirely within the sympathetic nervous system, with both afferent and efferent impulses passing through the gray ramus communicans to and from the stellate ganglion (p. 173). Coon and Rothman's experiments (29) on reflex sweating revealed that the reflex is abolished by dilutions of procaine of 1:1,000,000, a dilution which does not paralyze pain fibers but does paralyze sympathetic fibers. The axon-reflex sweating phenomenon can hardly be explained in any other way than by the assumption that an impulse ascends centripetally from the stimulated points of axon rami and then returns from the highest point of ramification to all the stimulated and nonstimulated rami of the very same axons. The axon-reflex sweating phenomenon clearly indicates that peripheral sympathetic sweat fibers may carry afferent impulses.

However, it should be emphasized that nothing definite is known about the afferent pathways of the general sweating reflex. Even if it should be shown in the future that the afferent path below the sympathetic ganglion is the same as the efferent one, its further course toward the diencephalon would still remain to be explored. The afferent pathways through which "mental" or "emotional" sweating is elicited are obviously sensory in nature (visual, auditory, cutaneous, etc.). "Emotional" sweating may

also develop from primary mental processes (for instance, by mental effort in arithmetic or by imagination without any demonstrable sensory stimulus), in which case no reflex process is postulated but a purely efferent impulse is operating.

E. THERMAL VERSUS SENSORY AND MENTAL STIMULI

Seemingly identical structures in the skin often display considerable differences in their responses to identical stimuli. Characteristic examples are the different effects of male sex hormones on scalp hair growth as contrasted with body hair (p. 626) or the selective pigmentogenic effect of estrogenic hormone on the areola of the nipples, midline of abdomen, and genitalia (p. 547). The selective responsiveness of palmar and plantar sweat glands to sensory (and mental) stimuli, as contrasted with their relatively sluggish response in thermoregulatory sweating, is a similar phenomenon. Kuno (90) dealt in great detail with this selectivity, and great significance has been attached to it in the literature on the galvanic skin reflex (113).

On moderate thermal stimulation the eccrine glands of the palms do not respond at all (60). On more intense heating, palms and soles are the last regions to show visible sweating (61). The axillary glands take an intermediate position. According to Kuno, they do respond to thermal stimulation much earlier than palmar glands; but they start to sweat relatively late, and, at the initial phases of heating, sweating is less pronounced there than on the body surface generally. Later in the course of thermoregulatory sweating, the flow of sweat from the axillae becomes quite profuse (90). Herrmann *et al.* (51) found much prompter response of axillary glands to thermal stimulation than Kuno did.

On mental and sensory stimulation, palmar and plantar sweat glands respond first and immediately; and if the mental stimulus is weak, no other glands respond (84). Sometimes, however, it is found that axillary glands respond to mental stimulation as

easily as do palmo-plantar glands (92). In this respect there seem to be considerable individual variations (90). Glands of the forehead also respond to mental stimuli, though the response is weaker than on palms (90). In cases of extreme mental excitement or violent sensory stimulation there is a general and profuse outbreak of sweat (90). Thus it is quite obvious that, although palmo-plantar and axillary glands are more responsive to sensory (and mental) stimuli and glands of all other parts more responsive to heat, the difference is only quantitative. Both types of glands apparently respond to both types of stimuli, but in different degrees.

For the axilla it is characteristic that, once the individual shows slight sweating response to mental stimuli, the response to thermal stimuli is also slight. Apparently the very same glands respond to both types of stimulation according to their innate ability to sweat. The problem of whether the apocrine glands respond only to mental stimulation is discussed on page 183.

Characteristically, Kuno (90) found that, at moderately warm environmental temperatures, moderately intense mental stimuli can cause general sweating much more easily than in the cold. This again shows the role of "conditioning" of sweat glands to any type of stimulation (p. 164). Conditioning is achieved also by habitual mechanical stimulation. Kuno (90) pointed out that palms and soles sweat most abundantly in pressure areas. One may speculate that this is the cause of the predominance of right-palm involvement, for instance, in *Trichophyton purpureum* infections of the palms (154).

Mental sweating is characterized by its quite immediate, sudden appearance, as contrasted with thermoregulatory sweating, which even in the case of sudden intense heating may have a latent period. This, of course, might be due to the fact that, in thermal sweating, time is required to raise the temperature of the skin (and blood) to the critical level, whereas mental sweating appears as suddenly as nerve conduction rate permits.

F. DISTURBANCES OF SWEAT SECRETION IN DISEASES OF THE CENTRAL NERVOUS SYSTEM

Cortical lesions in the parietal area often cause contralateral hyperidrosis (on the side of motor paralysis). It is not clear whether this is due to irritation of the diseased cortical center or to the release of cortical inhibition on subcortical centers (102). The latter is more probable.

That there is inhibition from superordinated centers seems to be well established by the phenomenon of drenching perspiration in severe transverse lesions of the spinal cord (108). This is part of a spinal mass-reflex, usually associated with piloerection, automatic bladder, spinal defense movements, etc., and is due to the liberation of intact spinal centers from the inhibitory influence of supraspinal levels. The reflex can be activated by a great number of visceral and somatic stimuli, such as distention of the bladder, change in the position of the bowels, pinpricks, thermal agents, etc. Even subthreshold stimuli may set up a constant excitatory state in the autonomous stump of the cord, with resulting mild "spontaneous" hyperidrosis. The relay of the reflex is in the lateral horn cells, and the efferent pathway is the same as that of physiological sweating. Thermoregulatory sweating can be elicited only in the segments above the transverse lesion, where it is mediated by the intact proximal part of the cord. Thus spinal sweating and thermoregulatory sweating are reciprocal, though not entirely so.

Observing transverse lesions in different levels of the cord, List and Pimenta (108) were able to map out *sympathetic dermatomes* which do not correspond to the well-known sensory dermatomes. The sympathetic dermatomes are larger and show more overlap.

Foerster (36) listed the following spinal segments supplying different regions of the skin surface with sweat fibers:

Head, neck, chest	C ₈ -D ₆	Lower part of trunk . . . Above	D ₉
Upper limbs . . .	D ₅ -D ₇	Lower limbs . . .	D ₉ -L ₂

According to List and Peet (104), the preganglionic sweat fibers supplying the different parts of the body originate in the following segments:

Head	D ₁ -D ₄	Trunk	D ₅ -D ₁₂
Upper extremi- ties	D ₄ -D ₇	Lower extremi- ties	D ₁₀ -L ₃

Hyperidrosis in the spinal mass-reflex may be so excessive that it becomes the predominant and most annoying symptom. The patient may become reconciled to motor paralysis, automatic bladder, and defense movements but cannot tolerate the drenching perspiration. Pre- or postganglionic sympathectomy is the only effective treatment (108).

If the efferent pathway of sweating is interrupted by circumscribed lesions of the central or the peripheral nervous system, circumscribed anidrosis results. Testing the localization, shape, and extent of the anidrotic area, either by direct testing of thermoregulatory sweating ability with indicators or by measuring skin resistance, has become an important diagnostic tool in neurology and neurosurgery. From the dermatological point of view, it is important to know that the activity of sweat glands surrounding anidrotic areas is increased. There is a so-called "border line" or "perilesionary" hyperidrosis in anidrosis of nervous origin (108), as well as in inflammatory skin lesions and in the sweat-retention syndrome (p. 281). The mechanism of this "compensatory" hyperidrosis is not understood, but possibly it is of axon-reflex origin (p. 172).

G. GUSTATORY SWEATING

Some normal persons, when tasting spicy or acid food or in some instances some particular food, such as chocolate, display some visible sweating over the lips, nose, nasolabial folds (Fig. 15) and sometimes even over the whole face and head. This sweating is accompanied by flushing of the involved areas. The response appears immediately on tasting the foodstuff and disappears rapidly on cessation of eating. In some cases the

phenomenon could be produced as a conditioned reflex by merely showing the specific offending foodstuff to the susceptible subject (106). This gustatory hyperidrosis is a rare condition in normals and is more frequently seen in hot climates.

However, it is fairly often seen in (1) diseases of the central nervous system, such as syringomyelia and encephalitis; (2) after cervical sympathetic section; and (3) after local trauma or disease, particularly in the region of the parotid gland following suppurative and cicatrizing processes. In the latter situation the "auriculotemporal syndrome" of Frey (38, 186) is a stereotypically localized gustatory hyperidrosis in the distribution of the auriculotemporal nerve and its anastomotic branches with the facial nerve (Fig. 16).

There have been many interpretations of this phenomenon; they were reviewed by Langenskiöld (94). It certainly is associated with the salivary reflex. The afferent limb of this reflex consists of gustatory sensory fibers of the glossopharyngeal nerve, and the efferent fibers are the secretory fibers of the glossopharyngeal nerve, chorda tympani, and auriculotemporal nerve. The salivary reflex is a cholinergic impulse, and it was assumed that excessive amounts of acetylcholine liberated by the parasympathetic cholinergic fibers of the efferent limb of the reflex may diffuse to sweat glands and stimulate them (106). This supposition is supported by the fact that in a great number of cases gustatory sweating appears in an area of the head in which the postganglionic sweat fibers have degenerated. If the sweat glands of the head are denervated, they are sensitized to acetylcholine and respond excessively. This exaggerated responsiveness was directly demonstrated by local injections of acetylcholine and pilocarpine into areas of gustatory hyperidrosis (94). In cases developing after traumatization of the parotid gland, it is assumed that the scar maintains a state of irritation of the non-sudoriferous cholinergic fibers which produce acetylcholine in excess. In this form sympathetic sudomotor fibers are not neces-

sarily damaged: reflex heat sweating of these areas may be normal.

In my opinion it is improbable that excess acetylcholine would diffuse from the parotid gland into the skin without being decomposed on its way there. However, the irritated nerve in the efferent limb of the salivary reflex also has cutaneous branches (e.g., in the auriculotemporal nerve), which, on



FIG. 15.—Gustatory sweating in a normal individual. Redrawn from List and Peet (103). (Reproduced by permission of Dr. List and the American Medical Association.)

irritation, liberate acetylcholine in the skin. Diffusion of excess acetylcholine within the skin to sweat glands is feasible. Excess acetylcholine may also be responsible for the flushing.

The hypothesis of diffusion of excess acetylcholine to sensitized denervated gland cells does not fit the observations of Haxton (50). This author observed gustatory hyperidrosis in a number of cases after cervical sympathectomies and found that the phenomenon can be completely abolished by lower thoracic sympathetic procaine block.

So far, this observation has remained unexplained.

A most peculiar case of unilateral gustatory hyperhidrosis on the left knee in an infant was recently reported by Mellinkoff

and Mellinkoff (118). Another recent observation that gustatory hyperhidrosis can be elicited by subcutaneous histamine injections and inhibited by antihistaminic drugs (182) is also difficult to interpret.

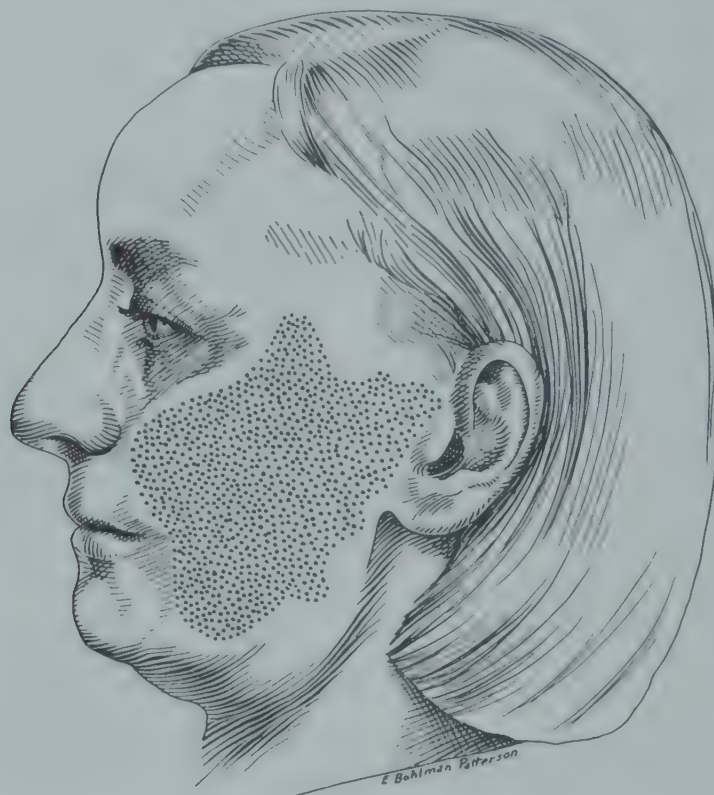


FIG. 16.—Area of vasodilatation in left auriculotemporal syndrome following abscess of the parotid gland. Redrawn from List and Peet (106). (Reproduced by permission of Dr. List and the American Medical Association.)

VIII. APOCRINE-GLAND SECRETION

A. GENERAL

Apocrine glands were first described by Horner in 1846 (58). They were rediscovered and called “apocrine” glands in 1922 by Schiefferdecker (160). In contrast to eccrine glands, which are independent appendages developing from the epidermis, apocrine glands develop from the follicular epithelium of the hair, as do sebaceous glands (177).

Racial differences in distribution were described, and it was claimed that the fewer the apocrine glands present, the greater the racial superiority (!) (160). This contention, however, was refuted when it was found that the individual variations in the apo-

crine/eccrine relationship in the pubic, mammary, and anal areas are so great that it can hardly be used for purposes of racial differentiation (200). Certainly, with the phylogenetic development of man, the apocrine glands, paralleling the gradual decline of the hair system, have become rudimentary in most places, while the eccrine glands have developed to a most perfect system. In most mammals eccrine glands occur only in the hairless regions, viz., the foot pads. In some apes they are scattered over the body surface (90). But in man they are present all over the surface and represent vitally important tools of heat regulation, enabling

him to live in extremely hot climates. Apocrine glands have taken an opposite course of development.

According to the classical view, apocrine glands take an intermediate position between true secretory (or merocrine) and holocrine glands. The luminal secretory cells

of true secretion and also discharging cellular particles (90, 121, 200).

This time-honored view was recently challenged by Shelley and Hurley (166, 168, 170). These authors—the first investigators to study pure apocrine secretion by collecting the product of single glands in glass

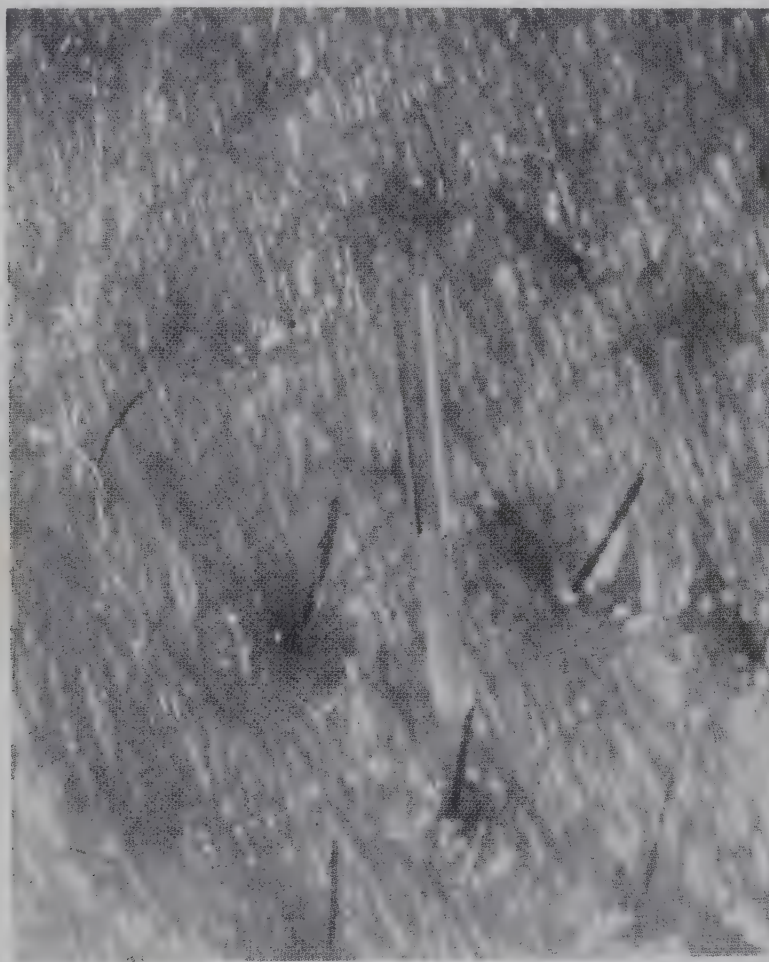


FIG. 17.—Apocrine sweat in axilla. Dried apocrine sweat appears as glistening caps about hair follicles. Observe capillary tube in hair follicle opening, containing whitish apocrine sweat. From Shelley and Hurley (170). (Reproduced by permission of the Williams & Wilkins Company.)

form lingual protuberances into the lumen; these projections are ruptured or torn off and discharged into the lumen with their protoplasm, globules, granules, and sometimes even nuclei. Most cells, however, are not ruined by this process. They re-form and discharge a fluid secretion similar to eccrine sweat until new cellular processes are formed. According to this view, supported solely by histological observations, apocrine glands function in two ways, being capable

micropipettes—found that apocrine sweat is a milky, whitish or grayish, sometimes yellowish, secretion, which, if not diluted by eccrine sweat, dries like glue to form a light-colored plastic solid (Fig. 17). It usually does not take the form of a spherical droplet, as eccrine sweat does, but forms a somewhat adherent cap on top of the orifice. In some instances it spreads over the perifollicular skin in a thin film without any convexity. The total volume of the “droplet” may be

as little as 0.001 ml. Consistency and color show great individual variations. Apocrine sweat droplets fluoresce (Fig. 18); eccrine droplets do not.

On adequate stimulation (epinephrine, emotional or painful stimuli) the droplet appears at the follicular opening after a latent period of 15 seconds or more. Once a droplet has been secreted, apocrine function ceases for several hours; during this refractory period adequate stimulation remains ineffective. Microanalysis of these droplets revealed the presence of protein, reducing sugars, ferric ion, and ammonia (170).

Shelley and Hurley (170) tentatively assumed that the secretion of milky droplets is

B. INFLUENCE OF AGE AND SEX

Like sebaceous glands and hair follicles, the apocrine glands undergo a spectacular development during puberty in both sexes (135). Axillary sweating in response to mental stimuli develops rather late. Children at the age of seven to nine years respond to mental stimuli with profuse sweating on palms and soles, but not with axillary sweat. At the same time, the axillae do sweat in response to thermal stimulation (90). Unfortunately, in these experiments eccrine and apocrine glands of the axillae were not clearly separated, and it is obvious that it would be erroneous to identify axillary sweating with apocrine sweating. The non-

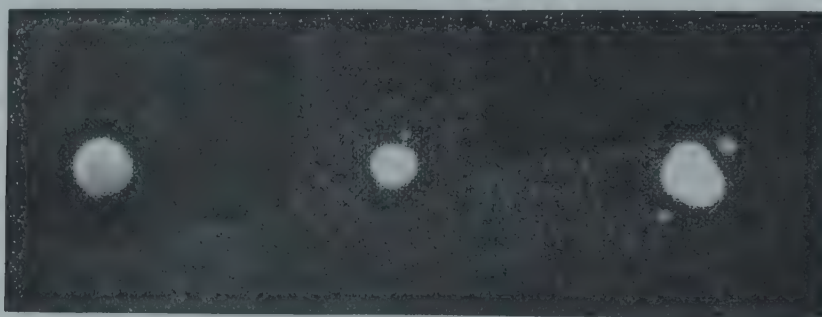


FIG. 18. Apocrine sweat fluorescence. Three droplets of dried apocrine sweat are shown under Wood's light. From Shelley and Hurley (170). (Reproduced by permission of the Williams & Wilkins Company.)

the only secretion type of apocrine glands. Their point is that the typical turbid apocrine sweat droplets can be seen to appear not only at the follicular orifices but also in between them; therefore, apocrine sweat should not be defined as sweat appearing in the follicular orifices. Conversely, the authors assumed that eccrine sweat might appear at the follicular openings, although they could not find anatomical evidence that eccrine glands actually open into follicular canals. But in nonapocrine-bearing hairy areas they could see "follicular eccrine" sweat. On this basis the authors are inclined to define any liquid, profusely flowing sweat as "eccrine," no matter whether it appears outside the follicular pore or on it. According to this view, apocrine secretion has only one, namely, a semiholocrine, aspect.

responsiveness of children's isolated axillary apocrine glands to adequate stimulation by epinephrine and sensory stimuli, however, has been confirmed (170).

There is a pronounced sex difference anatomically. The total number of apocrine glands is greater in women than in men (160, 88). Axillary sweating starts earlier in adolescent girls than in adolescent boys (112, 92). Secretory granules were said to be larger in the female (52).

The influences of the ovarian cycle, of pregnancy, and of the menopause have often been discussed (165, 190, 160, 110) but not actually measured. From some morphological data, it appears that during the menstrual cycle apocrine-gland function is highest premenstrually and lowest in the mid-menstruum (52). These differences, however, have not been found regularly

(82). Whereas earlier data on the effect of pregnancy had been contradictory, recently Cornbleet (30) presented supporting evidence for a depression of apocrine function during pregnancy, as was also found earlier by Richter (151). All this would be in line with what is known of hormonal effects on sebaceous glands: increased amounts of estrogens circulating in the blood depress glandular proliferation of sebaceous and apocrine glands. With the waning of sexual activity, the functioning of both kinds of glands seems to diminish, the diminution being connected with structural (involutional) changes (151, 20, 168). Analogous features of these two kinds of gland have been further emphasized by H. A. Brunsting (18), who correlates diseases of sebaceous glands and of apocrine glands as having similar genetic, hormonal, and infectious backgrounds.

Pana (132) finds a stricter correlation between gonadal development and the development, function, and involution of apocrine glands in the male than in the female. This does not parallel the events in sebaceous glands (p. 296).

There is an interesting analogy in the behavior of apocrine and mammary glands: not only do both glandular structures undergo involution simultaneously and show similar involutionary changes after the menopause, but if there are cystic changes in the mammary gland, they appear also in the apocrine glands (20). This is the case even in males with cystic gynecomastia (132).

C. ADEQUATE STIMULATION

In their recent work Shelley and Hurley (166, 168) found that apocrine glands do not respond to thermal stimulation but do respond promptly to mental stimuli. They believed that apocrine sweating observed in the heat cabinet was due to emotions (apprehension) rather than to heat, a conjecture which is difficult to prove. Kuno (90), although not collecting the secretion of single glands, as Shelley did, stated that in the axilla he obtained the very same point patterns to both types of stimulation, with the

points very close to the hairs in both instances. This observation, of course, does not invalidate Shelley's view. But Kuno (90) also claims that in children the same axillary glands respond to heat but not to emotions, an observation which, if correct, is hard to reconcile with Shelley's thesis.

Be that as it may, it appears that glands of the axilla in the adult respond, if at all, relatively late to general thermal stimulation, possibly later than all the eccrine glands except those on the palms and soles (90). On the other hand, according to Kuno (92, 90), mental stimuli elicit sweating in the axillae with the same ease as on the palms and soles (p. 177).

D. INNERVATION

In contrast to the situation with sebaceous glands (p. 303), autonomic innervation of apocrine glands is easily demonstrable. Reflex thermal sweating of apocrine glands was described by Aoki and Wada (7) in dogs, and emotional sweating by Kuno (90) and by Shelley (166) in man.

In some animals, such as horses and dogs, there seems to be a dual innervation of apocrine glands (123, 96, 7). In dogs, for instance, apocrine glands respond to local application of both epinephrine and acetylcholine, the minimal effective concentrations ranging from 10^{-6} to 10^{-8} for epinephrine and from 10^{-8} to 10^{-10} for acetylcholine (7). However, apocrine glands of sheep do not show cholinergic response (123), and the natural sweating of horses is not suppressed by atropine (123).

In man, Shelley (166) and Shelley and Hurley (168, 170) find that apocrine glands are exclusively adrenergic. Or, rather, they find that the expulsion of milky droplets at the follicular orifice, which they regard as the only function of the apocrine glands, is purely adrenergic. They do find fluid sweat at the follicular orifices on cholinergic stimulation, but they interpret this phenomenon as "eccrine follicular sweating" (170). Exceptionally, they also obtained "true" apocrine sweating on local injection of pilocarpine (170). (Effect of painful stimulation?)

They were of the opinion that the pure apocrine secretion can be best studied by local atropinization of the axillae, whereby eccrine glands are paralyzed and apocrine glands are left intact (168).

E. THE WORK OF SHELLEY AND HURLEY (166, 168, 170)

The interpretation of apocrine-gland function by Shelley and Hurley can be summarized as follows:

1. Apocrine-gland function has only one facet, namely, periodic secretion of a more or less viscous milky droplet. It is postulated that any profuse, fluid, nonviscous sweat flow is eccrine in origin. Whether sweat droplets appear at the follicular openings or not cannot be used as a criterion for distinguishing between apocrine and eccrine sweat.

2. Apocrine glands do not respond to thermal stimulation per se and do not take part in thermoregulation.

3. The secretion of the milky droplets by apocrine glands is purely adrenergic. Apocrine glands have no cholinergic innervation.

The assumption of "follicular eccrine sweating," of course, makes it difficult to arrive at any conclusion. The anatomical evidence has been rather convincing that eccrine and apocrine glands are anatomically clearly different structures, with distinctly separate localization, the apocrine glands emptying their secretion into the follicular canal and the eccrine glands having their own pores. If and when this anatomical evidence is disproved and if it ever becomes accepted that clear localizational separation is not possible, it is rather arbitrary—in the light of present evidence—whether one takes the view that all fluid sweat is eccrine or that apocrine glands have a dual function: periodic secretion of milky droplets and continuous secretion of fluid sweat.

Offhand, there are three main objections to the interpretation of Shelley and Hurley (166, 168, 170):

1. Mammals which have no eccrine glands on their trunk do secrete fluid profuse

sweat. These glands have a dual innervation (7).

2. In the anidrotic type of multiple ectodermal dysplasia in which the total number of eccrine glands is 5 per cent or less of the normal (34), there is profuse fluid thermal sweating exclusively at the sites of apocrine glands, viz., axillae and perimamillary region. The sweating of these areas is, in my experience, as profuse in the anidrotic patient as in normals under similar conditions.

3. The adrenergic response of the isolated apocrine glands is reminiscent of the adrenergic response of eccrine glands, mainly with regard to the refractory period which follows this response (p. 168). Whether or not it is accepted that this response is caused by myoepithelial contraction in the eccrine glands (p. 167), it certainly is justifiable to speak of a "dual" innervation of eccrine glands. The observations of Wada (188) strongly suggest that a similar situation prevails in the case of apocrine glands. In my view, as well as in that of other investigators,⁶ all available data, including those of Kuno and of Shelley and Hurley, as well as all histological findings justify the assumption that apocrine glands have a dual function: (a) expulsion of milky droplets by a semiholocrine mechanism, probably promoted by the adrenergic contraction of myoepithelia, and (b) secretion of fluid sweat on thermal and emotional stimulation, probably a cholinergic function. Cholinesterase was demonstrated histochemically in apocrine-gland secretory fibers by O. R. Aavik in my laboratory (Fig. 19).

In spite of these objections, Shelley and Hurley (personal communication) maintain that their interpretation is valid, for the following reasons:

1. Anatomically, it can be shown that in man eccrine ducts discharge into the "lip" of the hair follicles. One can see "follicular eccrine sweat" on nonapocrine-bearing sites of the body.

2. The clear fluid appearing at the follicle in the axilla corresponds chemically,

5. See particularly F. Herrmann, discussion on Shelley and Hurley (168).

functionally, and pharmacologically with eccrine sweat. On cholinergic stimulation, turbid apocrine secretion is never seen. If the clear fluid appearing at the follicular orifice in response to cholinergic stimulation is apocrine in origin, it should be mixed with turbid apocrine sweat (in my opinion, the absence of such a mixture can be very well explained by the assumption of a dual function).

3. The presence of a single type of secretory cell in the apocrine gland would seem to speak against the "dual secretion" theory (not if a combined holocrine-secretory function is assumed, in my opinion).

4. In the horse sweat glands can be demonstrated histologically which correspond morphologically with human eccrine glands. There is no turbid material in the horse's

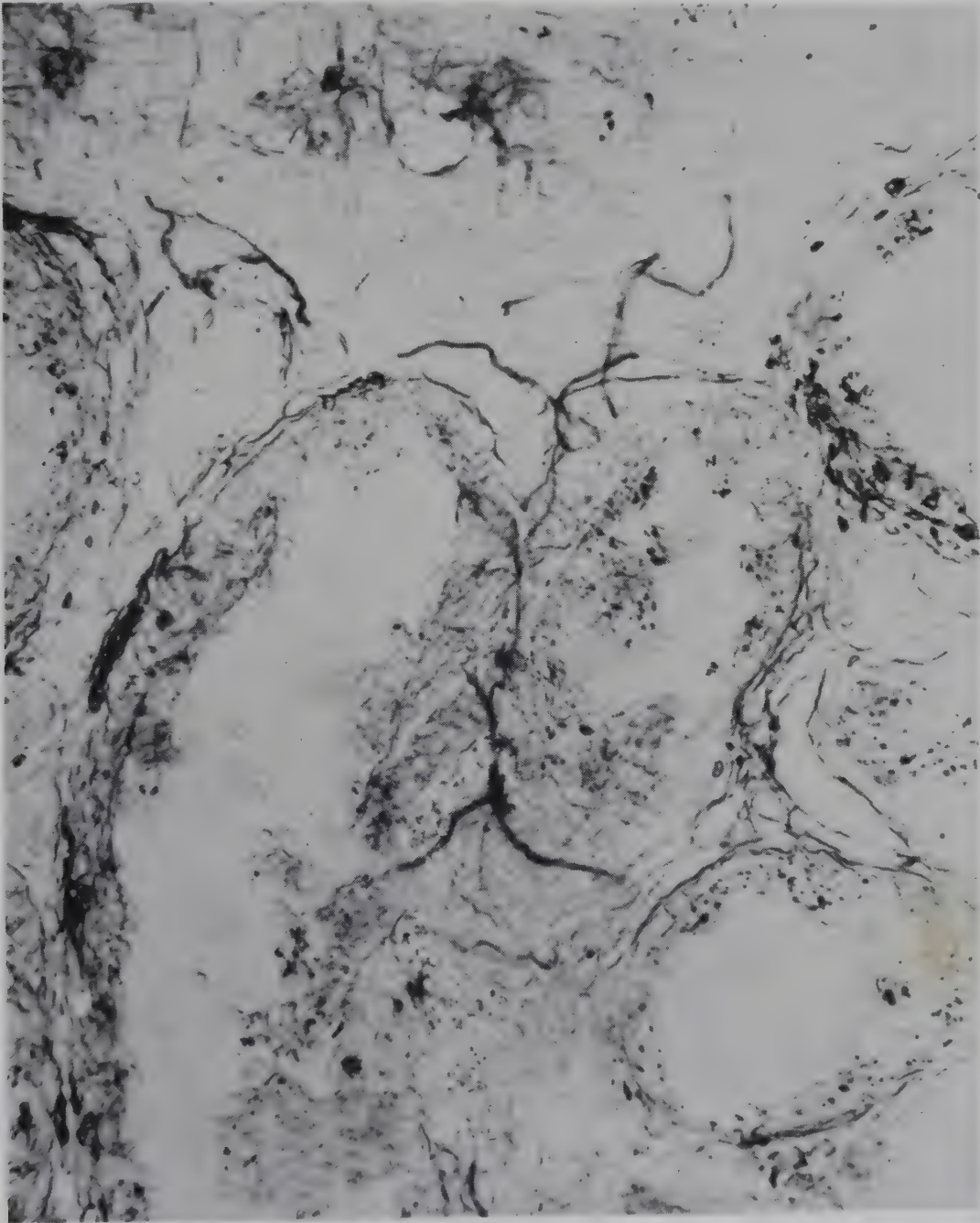


FIG. 19.—Histochemical demonstration of cholinesterase in nerve fibers of apocrine-gland walls. (Unpublished experiments of Dr. O. R. Aavik, Section of Dermatology, University of Chicago.)

sweat, or, if there is, it is produced by contamination of dander, according to veterinarians. In the genital region horses do produce a soapy or cloudy sweat which conceivably could be the product of true apocrine glands.

5. In patients with anidrotic congenital ectodermal defect the absence of eccrine glands in the axilla has not been directly demonstrated histologically.

6. Should the apocrine glands be able to secrete fluid sweat as eccrine glands do, they should contain glycogen as eccrine glands do. However, the glycogen content of apocrine glands is a controversial matter (see p. 191).

7. From a speculative (teleological) point of view, fluid, eccrine-like sweat flowing from axillary apocrine glands would be a completely superfluous function, because there are five to ten times more eccrine than apocrine glands in the axilla, and eccrine functions would be wasted in an atavistic structure such as the apocrine gland.

8. Most recently, in contrast to the findings of Aavik (Fig. 19), Hurley *et al.*, using a different histochemical technic, find that specific cholinesterase is absent around apocrine glands (H. J. Hurley, Jr., W. B. Shelley, and G. B. Koelle, The distribution of cholinesterases in human skin, with special reference to eccrine and apocrine sweat glands, *J. Invest. Dermat.*, **21**:139-47, 1953).

F. COMPOSITION OF APOCRINE SWEAT AND ITS ODORS

It has been known for a long time that apocrine sweat contains iron. Iron granules in the secretory part of apocrine glands were demonstrated histochemically by Homma in 1926 (56). Sometimes they can also be seen in the duct (19). Correspondingly, axillary hair shows faint traces of iron adhering to the shaft (19).

If there actually is considerable loss of iron through the skin (119), this seems to be primarily a function of apocrine glands, because relatively large amounts of iron are found only in turbid sweat. Adams *et al.* (2)

found 6-10 mg Fe/cc in originally opalescent sweat. After centrifugation, the clear supernatant fluid contained only 5 per cent of the original amount. These authors came to the conclusion that iron is lost through the skin not by secretion but by "desquamation." This "desquamative" process, however, seems to be greatly enhanced by heat sweating. Mitchell and Hamilton (119) find the dermal iron loss tremendously increased in hot humid climates. In such environments more than one-third of the total iron loss of the body is through the skin, whereas in a comfortable environment the participation of the skin amounts to only 13.4 per cent. All these findings, however, have lately been questioned by Whipple *et al.* (175) (p. 216).

As mentioned earlier, the presence of proteins, reducing sugars, and ammonia was demonstrated in the apocrine milky droplets by Shelley and Hurley (170). This was done by means of spot tests. Histochemical findings in apocrine-gland cells on the occurrence of glycogen, lipids, etc., are reviewed on pages 190 ff. The secretion of cholesterol by apocrine glands after pubertal development was reported by Richter (151). Most specimens of milky apocrine sweat droplets were found to fluoresce in ultraviolet light (170). Microscopically, orange-yellow fluorescent particles were observed in apocrine glands (19, 120). These were interpreted as being acetone-soluble lipid particles (19). It was emphasized that eccrine and apocrine sweat can be sharply distinguished on the basis of this fluorescence, because eccrine sweat never fluoresces (170). However, the fluorescent material might possibly also come from sebaceous glands which have a common orifice with the apocrine glands. I observed strong fluorescence of sebum fractions obtained from the hairy scalp, where apocrine glands are not present.

Coloration of sweat (chromidrosis) is much more frequently observed in the axillae than elsewhere, and therefore it may be assumed that apocrine glands can secrete chromogens or colored substances which are not present in eccrine sweat (116, 41, 137, 159, 170, 168). In a recent contribution

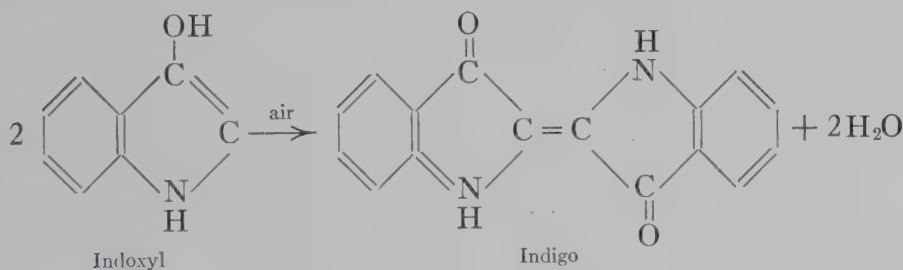
Shelley and Hurley (169), reviewing the literature and reporting on two cases of localized chromidrosis, came to the conclusion that secretion of colored sweat is an apocrine-gland function. In both their cases the colored droplets were turbid; they appeared at the follicular openings and in response to epinephrine but not to pilocarpine or heat; and they dried to form a glistening, glue-like mass. On local injection of epinephrine, a long latent period preceded the appearance of the droplets. Whereas their first case had colored sweat in the axilla, a typical site for apocrine glands, the site of localization of chromidrosis in the second case was in the face. In this latter case the authors postulated the presence of heterotopic apocrine glands, the occurrence of which was reported by Szodoray (180). In one of the cases the colored sweat was cultured, but no chromogenic bacteria were found.

The authors referred to the case of the

hippopotamus, which is famed for "sweating blood" and actually excretes red apocrine sweat when angered or otherwise excited. They properly pointed out that the possible relationship of this phenomenon to hysterical stigmata of localized "hematidrosis" is worthy of exploration. This is, no doubt, a most significant point.

Whether the authors are correct in assuming that colored sweat is *always* apocrine in origin remains to be proved. Palmar "bleeding" was reported in the case of Theresa of Konnersreuth (87). For phylogenetic reasons the occurrence of apocrine glands on the palms is improbable. It may very well be that adrenergic stimulation of the myoepithelia of eccrine glands may also produce colored sweat.

Formation of blue sweat was attributed to the secretion of indoxyl, a malodorous compound, which, on exposure to air, possibly as a result of bacterial activity, is oxidized into the blue dye, indigo (157):



Indoxyl leads to the discussion of the odorous substances which are secreted or excreted by apocrine glands in animals and man. Despite the great interest of scientists, industry, and laymen in this problem, no progress has been made in elucidating the nature of these substances in the last twenty-five years. In pure apocrine secretion obtained by cannulation of single pores, Shelley and Hurley (170) were unable to detect an odor in minute quantities, although in some subjects a definite odor appeared at the time of apocrine sweating. It should be emphasized that odorous substances are excreted not only by the apocrine but also by the sebaceous glands. Different fractions of human hair fat from the scalp prepared in the author's laboratory displayed "sweet-

ish," "bitter," and "dirty-comb" odors, respectively.

The information which existed twenty-five years ago is as follows (157): Skin odors belong in the category of "caprylic odors," which would indicate that they are due to the presence of free volatile fatty acids. The purest caprylic odor is found in the pubes, that of the axilla is more pungent, and that of the hairy scalp milder.

The odorous substances are sex-specific and obviously play an important role in sexual attraction and sexual life in the animal world. They also are more or less characteristic of different races, different families, and different individuals. Experiments with police-trained bloodhounds revealed that regional differences of smell in the same

individual are much greater than the smell of the same region from two different individuals (157).

It was assumed that, in addition to volatile fatty acids, hydroxyacids and ammonia also contribute to the odor. The presence of ammonia in pure apocrine sweat was recently demonstrated (170). Nothing is known about the occurrence of aromatic substances.

There has been much discussion on the possible role of bacterial activity in the formation of odorous substances on the skin surface, but this problem has never been experimentally investigated. I have found considerable amounts of free fatty acids in freshly excreted sebum of the hairy scalp and believe that in part they are primary products of glandular activity; but I have no direct proof for this belief. In the preen glands of water birds, where there is not much chance for bacterial invasion, free fatty acids are present. Sometimes the content of closed sebaceous cysts is very malodorous. But there is doubt whether this originates from the presence of low-boiling fatty acids (see p. 318) as was previously thought (157). It has been claimed that soaps medicated with antiseptic substances are more effective in suppressing "body odor" than soaps without such materials. Of course, people who do not wash themselves have a more obvious smell than clean people. Also, it is clear that bacterial activity contributes to the intensity and quality of smell in unclean people. But for people who remove apocrine and sebaceous secretion with warm water and soap at regular intervals several times weekly, it still has to be proved that bacterial activity contributes materially to their odor.⁶

It is an empirical fact that some individuals, despite scrupulous cleanliness, have intensely and unpleasantly smelling cutaneous excretions (bromidrosis). The substrates of this smell are unknown. Marchionini and Cerutti (115) believe that bromidrosis of the feet is connected with an alkaline reaction of sweat on the soles, because an alkaline milieu promotes the activity of *Bacterium graveolens* and *B. foetidum*. They found an acid sweat reaction in the arch of the sole, where sweat can evaporate, but alkaline reactions on the other parts, where sweat stagnates and decomposes. In cases of fallen arches the sweat on the whole surface of the soles is alkaline, because evaporation is not possible. According to these authors, orthopedic correction is needed, and treatment with acids might beneficially influence bromidrosis of the feet.

Whereas the odorous nature of apocrine and sebaceous secretions is common knowledge, little attention has been paid to the phenomenon that the strong odor of sebaceous and apocrine secretion of adolescents frequently becomes less intense after cutaneous glands have maximally developed and full maturity has set in. This is somewhat analogous to the spontaneous subsidence of acne vulgaris in the late twenties. Nor has the empirical fact been recorded that in adolescents not only sebaceous and apocrine glands but often also eccrine secretion of palms and soles has a more pungent smell than in childhood. This "bromidrosis" of palms and soles is mostly connected with hyperidrosis, but not necessarily so. Up to the present, no attention has been paid to the possible influence of sex hormones on eccrine glands.

IX. SWEAT SECRETION AND BLOOD FLOW

If during reflex sweating the circulation of a limb is occluded, sweating continues on

that limb with full intensity for 5–15 minutes or longer (90, 141, 142). Subsequently,

6. In their latest experiments Shelley *et al.* find that fresh apocrine sweat is odorless and sterile but acquires a typical acrid axillary odor by microbial action. Also they find that this microbial action can

be effectively counteracted by use of antiseptic soaps (W. B. Shelley, H. J. Hurley, Jr., and A. C. Nichols, Axillary odor, Arch. Dermat. & Syph., 68:430–46, 1953).

the number of functioning glands gradually decreases, secretion becomes intermittent, with decreasing peaks of the sweating cycle. Finally sweating ceases. This is apparently due to anoxemia of sweat nerve fibers, because the gland still responds to direct radiant heat (141, 142).

There is a relatively great independence of vasomotor and sweat impulses (p. 174). Thermogenic sweat, which appears on warm, flushed skin, has its counterpart in the "cold sweat" appearing in peripheral vascular collapse, with stasis of the cutaneous circulation and cold skin. Randall, Hertzman, and Ederstrom (145) measured sweat delivery and blood flow in different regions simultaneously. They came to the conclusion that, although there is a reasonable parallelism between sweating and blood-flow responses, significant discrepancies can be discovered in different skin areas, "thus failing to support the impression that in the zone of vasomotor regulation there is a smoothly operating decrease in vascular tone over the entire skin surface complementing the onset of generalized sweating." Thus, for instance, the face has a relatively high blood flow with low sweating rates, whereas on the dorsa of the hands and feet the sweating rates provide for evaporative heat loss in excess of the heat delivered by the blood. On the trunk, blood-flow rates and sweat rates are intermediate between the two other regions (144). It should be pointed out that the cheeks, while the main

site for blushing, are at the same time poor in sweat delivery. In contrast, the forehead sweats profusely and rarely blushes. Vasoconstriction and sweating in response to mental stimuli, to noises, and to deep inspiration are synchronous on finger pads but not on forearms (37).

The ease with which sweating, pilomotion, and vasoconstriction or vasodilatation can singly and separately be elicited by autonomic drugs (28) also speaks for a high degree of independence of these impulses and militates against the microscopic-anatomical view that the cutaneous autonomic nervous system is represented by a vast syncytium which connects functionally different organs, viz., blood vessels, sweat glands, pilomotor muscles, and even sebaceous glands with one another (176, 67, 128).

As first pointed out by Lewis (99) and fully confirmed in our laboratory (28, 29, 156), experimental physiological observations prove that all autonomic nerve fibers in the skin have widespread ramifications, crossing one another's pathways but never anastomosing with one another. It is not surprising that the dense maze of axon ramification appears microscopically as a syncytium which cannot be unraveled by the method of serial sections. The only way to follow the pathways of single fibers morphologically is by creating selective degeneration of certain kinds of fibers, leaving others intact, as was done on sensory fibers by Weddell (195) (p. 123).

X. THE MECHANISM OF ECCRINE SWEAT EXPULSION

In profuse sweating the pressure of the secreted fluid (250 mm. Hg) (16) is sufficient to cause an outpouring to the surface. In low-grade sweating, however, a special, expulsive mechanism is postulated, because sweat droplets may be seen rising and falling in the duct (90, 109) (Fig. 5, p. 157) and reaching the surface only occasionally. Apparently, there is some periodically acting extra force which presses the sweat droplets through the spiraling poral part of the duct to the surface.

The secretory portion of the sweat glands is closely invested with spindle-shaped "myoepithelial" cells, which are, in general, arranged with their long axes parallel to that of the tubule (19). They are inside the basement membrane. It is generally assumed that these are contractile elements (90, 109) and that their contraction promotes the delivery of sweat to the surface. Similarly, the weak rhythmic periodic contractions and relaxations of myoepithelium could be responsible for the rising and falling

levels within the ducts (109). There is reason to believe that these elements have an adrenergic innervation (p. 167).

In an entirely novel approach, Sulzberger, Herrmann, Keller, and Pisch (178) discovered still another possible mechanism of sweat expulsion. They found that anionic wetting agents and other electronegative compounds, when applied locally, increase the outpouring of sweat. Conversely, electropositive detergents reduce sweating to such a degree as to cause sweat retention,

with an eruptive response resembling *miliaria rubra* (p. 277). By means of vital staining they demonstrated that the whole spiral end-portion of the duct selectively stains with electropositive dyes, an indication that this portion has an electronegative charge. The conclusion was that the electronegativity at the distal end of the duct maintains an electrophysiological potential which promotes electro-osmotic passage of sweat to the skin surface.

XI. THE PROBLEM OF REABSORPTION

It has become commonplace to think of "ductal reabsorption" of sweat as analogous to tubular reabsorption in the kidney, although direct proof of such function of sweat glands is lacking. The first author to postulate reabsorption was Ackermann (1), with the hypothesis that probably only water is reabsorbed by the ducts and that this function is under the control of a double innervation: adrenergic impulses suppress it, whereas antidromic impulses of dorsal-root fibers, which allegedly inhibit visible sweat secretion (36), do so by promoting reabsorption of water. This view, however, was not supported by the experimental data presented.

Strong indirect evidence for reabsorption of water was presented by Lobitz and Mason (109) when they demonstrated that most constituents are much more concen-

trated in sweat which is secreted slowly ("intermittently") than in profusely secreted sweat. The supposition was that when sweat remains long in the duct, there is more opportunity for reabsorption of water. Indeed, the available data suggest that there is considerable water reabsorption from slowly secreted sweat. Reabsorption from profusely outpouring sweat still has to be demonstrated.

Conn (26) relates the decrease of salt concentration in sweat during acclimatization (p. 265) partly to reabsorption of salt, in analogy to kidney function. He calculated that if 1.9–3.2 gm. sodium chloride are given and 5–9 liters of sweat are lost every day, the limit of the sweat-gland ability to reabsorb salt is 0.25–0.35 gm. sodium chloride per liter.⁷

XII. HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS ON CHEMICAL CONSTITUENTS, STRUCTURE, AND FUNCTION OF SWEAT GLANDS

Basophilic dust and granules in the cytoplasm of eccrine- and apocrine-duct cells was tentatively identified as ribonucleoprotein. The particles disappear after treatment of histological sections with ribonuclease (19). Ample glycogen is present in the secretory cells of eccrine glands (157), disappearing from the secreting cells during activity (44, 202, 171) and reappearing after a rest period (202, 171) (see p. 466). Glyco-

gen depletion does not occur on "stimulation" with epinephrine or in atropinized areas, in spite of energetic heat stimulation (171). Thus glycogen degradation is clearly linked to the functional activity of the cells. The degradation of glycogen was thought to be the process possibly responsible for the high lactic acid content of sweat (p. 210).

7. Concerning reabsorption of water see also p. 193.

Glycogen persists for some time after death in the coil cells (187).

Apocrine-gland cells were said not to contain glycogen (170, 19), but its presence was later demonstrated (120). Physiologically, one should postulate its presence, because no difference was found in the lactic acid content of axillary and body sweat (130). In addition to glycogen, Montagna *et al.* (120) found Schiff-positive but diastase-resistant granules (mucopolysaccharides?) in secretory cells and in ductal lumina of eccrine and apocrine glands. In contrast to Bunting

"lipofuscin" or "wear-and-tear pigments" (*Abnutzungspigmente*). They seem to occur most commonly in the older age group (55). (3) A yellow pigment accompanies these droplets, which is soluble in acetone. (4) Some birefringent crystalline, acetone-soluble, sudanophilic material is present in the secretory cells of eccrine and apocrine glands. This material could be either fatty acids or cholesterol. It was found also in the luminae by Bunting *et al.* (19) but not by Shelley and Mescon (171). No birefringent material was found by Montagna *et al.* (120).



FIG. 20.—Drawing of a portion of the secretory segment of an apocrine sweat gland. Formalin-fixed, frozen section, stained with Sudan black B. From original of Fig. 1, H. Bunting, G. B. Wislocki, and E. W. Dempsey, *Anat. Rec.*, 100:69.

(19), they found glycogen also in the non-luminal epithelial cells of the ducts.

Four different kinds of lipid material were demonstrated histochemically in sweat-gland cells (19): (1) The cytoplasm contains a fine stippling of sudanophilic material, apparently representing mitochondria, since they coincide in size, number, and distribution. (2) Abundant sudanophilic droplets are scattered throughout the cytoplasm. They are abundant in eccrine and apocrine glands (Fig. 20). They are fluorescent (Fig. 21), plasmal-positive (containing an aldehyde or ketone group), insoluble in acetone, and soluble in pyridine. They might be identical with

There is accumulation of acid phosphatase in the capillary endothelia surrounding the sweat glands, in the basement membrane, in secretory cells, and, to a lesser degree, in myoepithelial cells (19, 171). This enzyme may play a role in glycogen degradation and synthesis. Also it was thought to be possibly responsible for the presence of phosphate ions in sweat (19). Shelley and Mescon (171) found alkaline phosphatase also in sweat-gland acini. Sweat-gland function does not alter the amount and distribution of alkaline phosphatase (171).

Iron is histochemically demonstrable in apocrine secretory epithelium, rarely also in

the secretions of these glands, but not at all in eccrine glands or their secretion (19). Axillary hair, as mentioned before, shows faint traces of iron adhering to the shaft.⁸

A remarkable structural change of eccrine glands was observed by Ring and Randall (152) on the paws of albino rats. On prolonged sciatic stimulation, there was a marked decrease in cell size associated with

duplicated by Shelley and Mescon (171) in their studies on human sweat glands. The difference in findings again could be due to differently intense stimulation.

A physiologically important structural problem has long been the nature of the wall of the intraepidermal portion of the duct. Early authors thought that this portion has no lining of its own but is bordered by rete cells of the epidermis, so that ma-



FIG. 21.—Auto-fluorescent droplets in the epithelium of the secretory segment of an apocrine sweat gland. Formalin fixation. Unstained frozen section. From original of Fig. 3, H. Bunting, G. B. Wislocki, and E. W. Dempsey, *Anat. Rec.*, **100**:77.

the heightened secretion. In man—with shorter and less intense stimulation—such an effect could not be obtained by Shelley and Mescon (171).

Sperling and Koppanyi (174), studying eccrine glands of cats stimulated by heat or by pilocarpine, found that the myoepithelia were reduced in size during activity and that the epidermal portion of the duct changed from a tight spiral to a loosely convoluted tube. They also found that functioning eccrine glands prevented reduced methylene blue from turning blue, while resting glands did not. Neither of these findings could be

terial could be freely exchanged between the duct and the intercellular spaces (185, 157, 194, 129). Melczer (117) was the first to show histochemically that such an exchange actually operates in the case of urea. When urea was infused intravenously, it entered the intraepidermal portion of the duct from the epidermis, whereas no urea was found in the deeper parts of the duct. Still, Melczer

⁸ For more recent histochemical observations see W. Montagna, H. B. Chase, and W. C. Lobitz, Jr., *Histology and cytochemistry of human skin. IV. The eccrine sweat glands*, *J. Invest. Dermat.*, **20**: 415–23, 1953.

held that the structural connection is not so free as Unna assumed (90). In 1939, F. Pinkus (136) presented morphological evidence that the intraepidermal duct wall has its own lining epithelium which is easily distinguished from rete cells. Holyoke and Lobitz (55) supported this view. Of course, physiologically the permeability of these specific cells may still be much greater than that of the lining cells in the lower parts of

the ducts, and therefore a greater exchange of constituents of intracellular canals in the rete is still possible. This problem is important from the point of view of reabsorption of sweat constituents (p. 190), as well as for the sweat-retention syndrome (pp. 270 ff.), particularly concerning the controversial question of whether the duct wall has to rupture in order to permit seepage of sweat into the rete cell (p. 272).

BIBLIOGRAPHY

1. ACKERMANN, A. Studien zur Physiologie der Schweißdrüsen; zur Pharmakologie und Funktionsweise der Schweißdrüsen, *Dermatologica*, **79**:151-74 and 219-36, 1939.
2. ADAMS, W. S.; LESLIE, A.; and LEVIN, M. H. The dermal loss of iron, *Proc. Soc. Exper. Biol. & Med.*, **74**:46-48, 1950.
3. ADOLPH, E. F. The initiation of sweating in response to heat, *Am. J. Physiol.*, **145**:710-15, 1946.
4. ALBERT, R. E., and PALMES, E. D. Pulsative evaporative rate from small skin area as measured by an infra-red gas analyzer, *Federation Proc.*, **8**:1-2, 1949.
5. ———. Evaporative rate patterns from small skin areas as measured by an infra-red gas analyzer, *J. Appl. Physiol.*, **4**:208-14, 1951.
6. ALVERDES, K. Die apokrinen Drüsen im Vestibulum nasi des Menschen, *Ztschr. f. mikr.-anat. Forsch.*, **28**:609-43, 1932.
7. AOKI, T., and WADA, M. Functional activity of the sweat glands on the hairy skin of the dog, *Science*, **114**:123-27, 1951.
8. ARNOLD, H. L., JR. The sweat response to intradermally injected mecholyl; preliminary report of its possible use in the diagnosis of leprosy, *Proc. Staff Meet. Clin. Honolulu*, **11**:75-81, 1945.
9. ———. The intradermal mecholyl test for anhidrosis; a diagnostic aid in leprosy, *Internat. J. Leprosy*, **16**:335-46, 1948.
10. BARBOUR, H. G. Die Wirkung unmittelbarer Erwärmung und Abkühlung der Wärmezentra auf die Körpertemperatur, *Arch. f. exper. Path. u. Pharmacol.*, **70**:1-26, 1912.
11. ———. The heat regulating mechanism of the body, *Physiol. Rev.*, **1**:295-326, 1921.
12. BARNETT, A. J. Sweating in man from the intradermal injection of nor-adrenaline, *Nature*, **167**:482-83, 1951.
13. BAZETT, H. C. The effect of temperature on the skin; a review, *J. Invest. Dermat.*, **1**:413-25, 1938.
14. BEATON, L. E., and LENINGER, C. R. Spinal distribution of the thermoregulatory pathways in the monkey, *J. Neurophysiol.*, **6**:37-38, 1943.
15. BEATON, L. E.; LENINGER, C. R.; and MCKINLEY, W. A. Thermoregulatory pathways in the cat brain stem, *J. Neurophysiol.*, **6**:29-35, 1943.
16. BEST, C. H., and TAYLOR, N. B. The physiological basis of medical practice. ("University of Toronto texts in applied physiology.") 5th ed. Baltimore: Williams & Wilkins Co., 1950.
17. BICKFORD, R. G. The mechanism of local sweating in response to faradism, *Clin. Sc.*, **3**:337-41, 1938.
18. BRUNSTING, H. A. Hidradenitis and other variants of acne, *Arch. Dermat. & Syph.*, **65**:303-15, 1952.
19. BUNTING, H.; WISLOCKI, G. B.; and DEMPSEY, E. W. The chemical histology of human eccrine and apocrine sweat glands, *Anat. Rec.*, **100**:61-77, 1948.
20. BUSCHKE, W. Cystenmamma und Axillarorgan (auf Grund von Untersuchungen an apokrinen Achselschweißdrüsen im Klimakterium), *Arch. f. Gynäk.*, **152**:431-46, 1933.
21. CANNON, W. B. A law of denervation, *Am. J. M. Sc.*, **198**:737-50, 1939.
22. CANNON, W. B., and ROSENBLUETH, A. Autonomic neuro-effector systems. New York: Macmillan Co., 1937.
23. CHALMERS, I. M., and KEELE, C. A. The

- nervous and chemical control of sweating, *Brit. J. Dermat.*, **64**:43-54, 1952.
24. CLARK, G.; MAGOUN, H. W.; and RANSON, S. W. Hypothalamic regulation of body temperature, *J. Neurophysiol.*, **2**:61-80, 1939.
 25. CLAUSHEN, A. Mikroskopische Untersuchungen über die Epidermalgebilde am Rumpfe des Hundes mit besonderer Berücksichtigung der Schweißdrüsen, *Anat. Anz.*, **77**:81-97, 1933.
 26. CONN, J. W. The mechanism of acclimatization to heat. In: *Advances in internal medicine*, **3**:373-93. New York: Interscience Publishers, Inc., 1949.
 27. COON, J. M., and ROTHMAN, S. The nature of the sweat response to drugs with nicotine-like action, *Proc. Soc. Exper. Biol. & Med.*, **42**:231-33, 1939.
 28. ———. The nature of the pilomotor response to acetylcholine. Some observations on the pharmacodynamics of the skin, *J. Pharmacol. & Exper. Therap.*, **68**:301-11, 1940.
 29. ———. The sweat response to drugs with nicotine-like action, *ibid.*, **73**:1-11, 1941.
 30. CORNBLEET, T. Pregnancy and apocrine gland disease: hidradenitis, Fox-Fordyce disease, *Arch. Dermat. & Syph.*, **65**:12-19, 1952.
 31. DALE, H. H. Nomenclature of fibres in the autonomic system and their effects, *J. Physiol.*, **80**:10P-11P, 1933.
 32. DALE, H. H., and FELDBERG, W. The chemical transmission of secretory impulses to the sweat glands of the cat, *J. Physiol.*, **82**:121-28, 1934.
 33. ERLANGER, J., and GASSER, H. S. Electrical signs of nervous activity. Philadelphia: University of Pennsylvania Press, 1937.
 34. FELSHER, Z. Hereditary ectodermal dysplasia. Report of a case, with experimental study, *Arch. Dermat. & Syph.*, **49**:410-14, 1944.
 35. FILEHNE, W. Zur Lehre von der Wärme-regulation, *Arch. f. Physiol.*, pp. 551-58, 1910.
 36. FOERSTER, O. Die Symptomatologie der Schussverletzungen der peripheren Nerven, *Handb. f. Neurol.*, p. 1485, 1929. Quoted by KUNO (90).
 37. FRANKE, F. E.; RANDALL, W. C.; SMITH, D. E.; and HERTZMAN, A. B. Vasomotor and sudomotor patterns in the skin of the fingers and forearm, *Federation Proc.*, **6**:105, 1947.
 38. FREY, L. Le syndrome du nerf auriculo-temporal, *Rev. neurol.*, **2**:97-104, 1923.
 39. GIBSON, T. E., and SHELLEY, W. B. Sexual and racial differences in the response of sweat glands to acetylcholine and pilocarpine, *J. Invest. Dermat.*, **11**:137-42, 1948.
 40. GOODMAN, L. S., and GILMAN, A. The pharmacological basis of therapeutics: a textbook of pharmacology, toxicology and therapeutics for physicians and medical students. New York: Macmillan Co., 1949.
 41. GREIG, D. M. The analogy of black colostrum to melanhidrosis, with some remarks on coloured milk and coloured sweat, *Edinburgh M. J.*, **37**:524-44, 1930.
 42. GROSSMAN, M. I.; JANOWITZ, H. D.; and SONNENSCHNEIN, R. R. Action of neurohumoral agents in the human skin, *Federation Proc.*, **8**:63, 1949.
 43. GROTH, W. Der Verlauf des Drüsen-schlauches in den a-Drüsen der Achselhaut des Menschen, *Ztschr. f. mikr.-anat. Forsch.*, **38**:627-34, 1935.
 44. GUALDI, A., and BALDINO, N. Ricerche sul metabolismo per variazioni della temperatura locale dei tessuti; il contenuto in glicogeno e in acido lattico della cute e dei muscoli raffreddati, *Riv. di pat. sper.*, **5**:318-22, 1930.
 45. GUTTMANN, L. Die Schweißsekretion des Menschen in ihren Beziehungen zum Nervensystem, *Ztschr. f. d. ges. Neurol. u. Psychiat.*, **135**:1-48, 1931.
 46. GUTTMANN, L., and LIST, C. F. Zur Topik und Pathophysiologie der Schweißsekretion, *Ztschr. f. d. ges. Neurol. u. Psychiat.*, **116**:504-36, 1928.
 47. HAIMOVICI, H. Evidence for an adrenergic component in the nervous mechanism of sweating in man, *Proc. Soc. Exper. Biol. & Med.*, **68**:40-41, 1948.
 48. ———. Evidence for adrenergic sweating in man, *J. Appl. Physiol.*, **2**:512-21, 1950.
 49. HARDY, J. D.; MILHORAT, A. T.; and DU BOIS, E. F. Basal metabolism and heat loss of young women at temperatures from 22° C. to 35° C., *J. Nutrition*, **21**:383-404, 1941.
 50. HAXTON, H. A. Gustatory sweating, *Brain*, **71**:16-25, 1948.
 51. HERRMANN, F.; PROSE, P. H.; and SULZBERGER, W. B. Studies on sweating. V. Studies of quantity and distribution of

- thermogenic sweat delivery to the skin, *J. Invest. Dermat.*, **18**:71-86, 1952.
52. HERZENBERG, H. Neue Beiträge zur Lehre von den apokrinen Schweißdrüsen, *Virchows Arch. f. path. Anat.*, **266**:422-55, 1927.
 53. HILL, L. Cooling and warming of the body by local application of cold and heat, *Proc. Physiol. Soc., London*, **54**:137, 1920-21.
 54. HOEPKE, H. Die Drüsen der Haut, *Handb. d. Mikr.-Anat. d. Menschen*, **3**:55-66. Berlin: J. Springer, 1927.
 55. HOLYOKE, J. B., and LOBITZ, W. C., JR. Histologic variations in the structure of human eccrine sweat glands, *J. Invest. Dermat.*, **18**:147-67, 1952.
 56. HOMMA, H. On apocrine sweat glands in white and Negro men and women, *Bull. Johns Hopkins Hosp.*, **38**:365-71, 1926.
 57. HORN, G. Formentwicklung und Gestalt der Schweißdrüsen der Fusssohle des Menschen, *Ztschr. f. mikr.-anat. Forsch.*, **38**:318-29, 1935.
 58. HORNER, W. E. Quoted in: The apocrine glands (editorial), *Brit. M. J.*, **1**:574, 1939.
 59. HYNDMAN, O. R., and WOLKIN, J. The pilocarpine sweating test. I. A valid indicator in differentiation of preganglionic and postganglionic sympathectomy, *Arch. Neurol. & Psychiat.*, **45**:992-1006, 1941; see also pp. 446-67.
 60. IKEUCHI, K. Quoted by KUNO (90).
 61. IKEUCHI, K., and KUNO, Y. On the regional differences of the perspiration on the surface of the human body, *J. Orient. Med.*, **7**:67-89 and 106-7, 1927.
 62. ISSEKUTZ, B., JR.; HETÉNYI, G., JR.; and DIÓSY, A. The physiology of sweat secretion, *Arch. internat. de pharmacodyn. et de therap.*, **83**:133-42, 1950.
 63. JANOWITZ, H., and GROSSMAN, M. I. Blocking action of tetraethylammonium on axon reflexes in the human skin, *Science*, **109**:16, 1949.
 64. ———. The response of the sweat glands to some locally acting agents in human subjects, *J. Invest. Dermat.*, **14**:453-58, 1950.
 65. ———. An exception to Cannon's law, *Experientia*, **7**:275, 1951.
 66. JANOWITZ, H.; SONNENSCHNEIN, R. R.; and GROSSMAN, M. I. Adrenotropic receptors of the human skin, *J. Invest. Dermat.*, **12**:205-6, 1949.
 67. JOHN, F. Zur mikroskopischen Anatomie der Gefäß- und Schweißdrüsenerven in der menschlichen Haut, *Ztschr. f. Zellforsch. u. mikr. Anat.*, **30**:297-320, 1940.
 68. JORES, A. Perspiratio insensibilis. I, *Ztschr. f. d. ges. exper. Med.*, **71**:170-85, 1930.
 69. ———. Perspiratio insensibilis. II. Über die Beziehung zwischen renaler und extrarenaler Wasserausscheidung, *ibid.*, **74**:757-68, 1930.
 70. ———. Perspiratio insensibilis. III, *ibid.*, **77**:734-42, 1931.
 71. JÜRGENSEN, E. Mikrobeobachtungen der Schweißsekretion der Haut des Menschen. I, *Deutsche Ztschr. f. klin. Med.*, **144**:193-201, 1924.
 72. ———. Mikrobeobachtungen der Schweißsekretion der Haut des Menschen. II. Funktionsprüfungen, Methode und Begründung; allgemeiner Überblick; praktische Anwendung, *ibid.*, pp. 248-57, 1924.
 73. ———. Mikrobeobachtungen der Schweißsekretion der Haut des Menschen. III, *ibid.*, **149**:157-67, 1925.
 74. ———. Mikrobeobachtungen der Schweißsekretion der Haut des Menschen. IV. Vergleichende Reizreaktionen unter verschiedener örtlicher Hautdurchblutung, *ibid.*, **155**:342-52, 1927.
 75. ———. Mikrobeobachtungen der Schweißsekretion der Haut des Menschen. V. Atropinstudien, *ibid.*, **165**:165-79, 1929.
 76. KAHN, D., and ROTHMAN, S. Sweat response to acetylcholine, *J. Invest. Dermat.*, **5**:431-44, 1942.
 77. KAWAHATA, A., and SAKAMOTO, H. Some observations on sweating of the Aino, *Jap. J. Physiol.*, **2**:166-69, 1951.
 78. KELLER, A. D. Descending nerve fibers subserving heat maintenance functions coursing with cerebrospinal tracts through the pons, *Am. J. Physiol.*, **154**:82-86, 1948.
 79. KENNARD, M. A. Vasomotor disturbances resulting from cortical lesions, *Arch. Neurol. & Psychiat.*, **33**:537-45, 1935.
 80. KENNARD, M. A.; VIETS, H. R.; and FULTON, J. F. The syndrome of the premotor cortex in man; the impairment of skilled movements, forced gasping, spasticity and vasomotor disturbance, *Brain*, **57**:69-84, 1934.
 81. KISIN, E. E. A method of pharmacological study of the sweating function of the skin in situ, *Vestnik venerol. i dermat.*, **5**:27-

- 31, 1948. Quoted in Chem. Abstr., **43**: 2323c, 1949.
82. KLAAR, J. Zur Kenntnis des weiblichen Axillarorgans beim Menschen, *Klin. Wchnschr.*, **39**:127-31, 1926.
83. KOPPANYI, T. Hexadienol, a locally acting diaphoretic and a new diagnostic agent, *J. Am. Pharm. A.*, **34**:221-24, 1945.
84. KOSAKA, T. Quoted by KUNO (90).
85. KRAUSE. Wagners Handwörterbuch der Physiologie, **2**:131, 1844. Quoted by KUNO (90).
86. KREDEL, F. E., and PHEMISTER, D. B. Recovery of sympathetic nerve function in skin transplants, *Arch. Neurol. & Psychiat.*, **42**:403-12, 1939.
87. KRÖNER, W. Das Rätsel von Konnersreuth und Wege zu seiner Lösung; Studie eines Parapsychologen; mit einem Geleitwort v. Hans Driesch, Verlag d. Aerztl. Rundschau. Munich: Otto Gmelin, 1927.
88. KUDO, K. Quoted by KUNO (90).
89. KÜSTERMANN, W. Über die anatomische Beziehung zwischen Schweissdrüsenausführungsgang und Papillarsystem. Diss., Hamburg, 1932. Quoted in *Zentralbl. f. Haut- u. Geschlechtskr.*, **44**:528, 1933.
90. KUNO, Y. The physiology of human perspiration. London: J. & A. Churchill, Ltd., 1934.
91. ———. Variations in secretory activity of human sweat glands, *Lancet*, **1**:299-303, 1938.
92. KUO, K. W. Quoted by KUNO (90).
93. KURÉ, K.; OKINAKA, S.; MAEDA, S.; and KATO, H. Studien über die Schweissdrüseninnervation, *Arch. f. d. ges. Physiol.*, **237**:40-53, 1936.
94. LANGENSKIÖLD, A. Gustatory local hyperhidrosis following injuries in the parotid region, *Acta chir. Scandinav.*, **93**:294-306, 1946.
95. LANGLEY, J. N. The secretion of sweat. Supposed inhibitory nerve fibers of the posterior nerve roots; secretion after denervation, *J. Physiol.*, **56**:110-19, 1922.
96. LANGLEY, J. N., and BENNETT, S. Action of pilocarpine, arecoline and adrenaline on sweating in the horse, *J. Physiol.*, **57**: lxxi-lxxii, 1923.
97. LANGLEY, J. N., and UYENO, K. The secretion of sweat. II. The effect of vasoconstriction and of adrenaline, *J. Physiol.*, **56**:206-26, 1922.
98. LANGSWORTHY, O. R., and RICHTER, C. P. The influence of efferent cerebral pathways upon the sympathetic nervous system, *Brain*, **53**:178-93, 1930.
99. LEWIS, T. Experiments relating to cutaneous hyperalgesia and its spread through the somatic nerves, *Clin. Sc.*, **2**: 373-417, 1936.
100. LEWIS, T., and LANDIS, E. M. Some physiological effects of sympathetic ganglionectomy on the human being and its effect in a case of Raynaud's malady, *Heart*, **15**:151-76, 1930.
101. LEWIS, T., and MARVIN, H. M. Observations upon a pilomotor reaction in response to faradism, *J. Physiol.*, **64**:87-106, 1927.
102. LINDER, F. Über den Einfluss der Hirnrinde auf die Schweisssekretion (zur Frage der vegetativen Hemiplegie), *Deutsche Ztschr. f. Nervenhe.*, **157**:86-94, 1947.
103. LIST, C. F., and PEET, M. M. Sweat secretion in man. I. Sweating responses in normal persons, *Arch. Neurol. & Psychiat.*, **39**:1228-37, 1938.
104. ———. Sweat secretion in man. II. Anatomic distribution of disturbances in sweating associated with lesions of the sympathetic nervous system, *ibid.*, **40**:27-43, 1938.
105. ———. Sweat secretion in man. III. Clinical observations on sweating produced by pilocarpine and mecholyl, *ibid.*, pp. 269-90.
106. ———. Sweat secretion in man. IV. Sweat secretion of the face and its disturbances, *ibid.*, pp. 443-70.
107. ———. Sweat secretion in man. V. Disturbances of sweat secretion with lesions of the pons, medulla and cervical portion of the cord, *ibid.*, **42**:1098-1127, 1939.
108. LIST, C. F., and PIMENTA, A. DE M. Sweat secretion in man; spinal reflex sweating, *Arch. Neurol. & Psychiat.*, **51**:501-7, 1944.
109. LOBITZ, W. C., JR., and MASON, H. L. Chemistry of palmar sweat. VII. Discussion of studies on chloride, urea, glucose, uric acid, ammonia-nitrogen and creatinine, *Arch. Dermat. & Syph.*, **57**:907-15, 1948.
110. LOESCHKE, H. Über zyklische Vorgänge in den Drüsen des Achselhöhlenorgans und ihre Abhängigkeit vom Sexualzyklus des Weibes, *Virchows Arch. f. path. Anat.*, **255**:283-94, 1925.
111. LORINCZ, A. L. Personal communication.

112. LÜNEBURG. Beiträge zur Entwicklung und Histologie der Knäueldrüsen in der Achselhöhle des Menschen, Inaug. diss., Rostock, 1902. Quoted by KUNO (90).
113. McCLEARY, R. A. The nature of the galvanic skin response, *Psychol. Bull.*, **47**: 97-113, 1950.
114. MAGOUN, H. W.; HARRISON, F.; BROBECK, J. R.; and RANSON, S. W. Activation of heat loss mechanisms by local heating of the brain, *J. Neurophysiol.*, **1**:101-14, 1938.
115. MARCHIONINI, A., and CERUTTI, P. Physikalisch-chemische Untersuchungen zur Pathogenese der Bromhidrosis pedum, *Arch. f. Dermat. u. Syph.*, **166**:354-62, 1932.
116. MAYR, J. K. Die Erkrankungen der Schweissdrüsen. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **13**:1-47. Berlin: J. Springer, 1932.
117. MELCZER, N. Experimentelle Untersuchungen über die Ausscheidung des Carbamids, *Arch. f. Dermat. u. Syph.*, **150**:235-39, 1926.
118. MELLINKOFF, S. M., and MELLINKOFF, J. Gustatory hyperhidrosis of the left knee, *J.A.M.A.*, **142**:901-2, 1950.
119. MITCHELL, H. H., and HAMILTON, T. S. The dermal excretion under controlled conditions of nitrogen and minerals in human subjects, with particular reference to calcium and iron, *J. Biol. Chem.*, **178**:345-61, 1949.
120. MONTAGNA, W.; CHASE, H. B.; and HAMILTON, J. B. The distribution of glycogen and lipids in human skin, *J. Invest. Dermat.*, **17**:147-57, 1951.
121. MONTAGNA, W.; NOBACK, C. R.; and ZAK, F. G. Pigment, lipids, and other substances in the glands of the external auditory meatus of man, *Am. J. Anat.*, **83**:409-36, 1948.
122. MOOG, O. Der Einfluss von Pilokarpin, Atropin und Adrenalin auf die unmerkliche Hautwasserabgabe *Arch. f. exper. Path. u. Pharmacol.*, **98**:75-90, 1923.
123. MUTO, K. Über die Wirkung des Adrenalins auf die Schweisssekretion; zugleich ein Beitrag zur Kenntnis der doppelsinnigen Innervation der Schweissdrüsen, *Mitt. a. d. med. Fak. d. k. Univ. zu Tokyo*, **15**: 365-86, 1916.
124. MYERSON, A.; LOMAN, J.; and RINKEL, M. Human autonomic pharmacology; general and local sweating produced by acetyl- β -methyl-choline chloride (mecholy), *Am. J. M. Sc.*, **194**:75-79, 1937.
125. NAGASHIMA, T. Über Ceruminaldrüse und Cerumen, besonders die Beziehung derselben zu Osmidrosis axillaris bei Japanern, *Jap. J. Dermat. & Urol.*, **36**:118-19, 1934.
126. NETSKY, M. G. Studies on sweat secretion in man, *Arch. Neurol. & Psychiat.*, **60**: 279-87, 1948.
127. OHARA, K. Studies on the oxygen consumption of human skin tissues with special reference to that of sweat glands, *Jap. J. Physiol.*, **2**:1-8, 1951.
128. ORMEA, F. On the problem of the relations between the innervation of the sweat glands and of other organs of the human skin, *Dermatologica*, **101**:157-66, 1950.
129. ORMSBY, O. S., and MONTGOMERY, H. Diseases of the skin. 7th ed. Philadelphia: Lea & Febiger, 1948.
130. OTTENSTEIN, B. Beitrag zur Chemie des Schweisses, *Arch. f. Dermat. u. Syph.*, **191**:116-22, 1949.
131. PALMES, E. D. An apparatus and method for the continuous measurement of evaporative water loss from human subjects, *Rev. Scient. Instruments*, **19**:711-17, 1948.
132. PANA, C. Ricerche sulle variazioni strutturali delle ghiandole apocrine ascellari in relazione allo stato della ghiandole sessuali e della mamella, *Sperimentale, Arch. d. biol.*, **88**:850-907, 1934.
133. PATON, W. D. M. The paralysis of autonomic ganglia, with special reference to the therapeutic effects of ganglion-blocking drugs, *Brit. M. J.*, **1**:773-78, 1951.
134. PATTON, H. D. Effect of autonomic blocking agents on sweat secretion in cat, *Proc. Soc. Exper. Biol. & Med.*, **70**:412-14, 1949.
135. PINKUS, F. Die normale Anatomie der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1**:1-378. Berlin: J. Springer, 1927.
136. PINKUS, H. Notes on the anatomy and pathology of the skin appendages. I. The wall of the intraepidermal part of the sweat duct, *J. Invest. Dermat.*, **2**:175-86, 1939.
137. Pozzo, A. Cromidrosi rossa ascellare legata a iniezioni di jodo bismutato di chinino, *Gior. veneto di sc. med.*, **8**:486-89, 1934.
138. RANDALL, W. C. Sweat gland activity and changing patterns of sweat secretion, on

- the skin surface, *Am. J. Physiol.*, **147**:391-98, 1946.
139. RANDALL, W. C. Quantitation and regional distribution of sweat glands in man, *J. Clin. Investigation*, **25**:761-67, 1946.
 140. ———. Local sweat gland activity due to direct effects of radiant heat, *Am. J. Physiol.*, **150**:365-71, 1947.
 141. ———. Reflex sweating response and the influence of arterial occlusion upon sweat gland activity, *Federation Proc.*, **6**:183-84, 1947.
 142. RANDALL, W. C.; DEERING, R.; and DOUGHERTY, I. Reflex sweating and the inhibition of sweating by prolonged arterial occlusion, *J. Appl. Physiol.*, **1**:53-59, 1948.
 143. RANDALL, W. C.; DOUGHERTY, I.; and DEERING, R. Sweating patterns in the skin following injections of mecholyl, *Am. J. Physiol.*, **151**:576-80, 1948.
 144. RANDALL, W. C., and HERTZMAN, A. B. Relations between blood flow, skin temperature, evaporation rates and sweating in various skin regions, *Federation Proc.*, **8**:129-30, 1949.
 145. RANDALL, W. C.; HERTZMAN, A. B.; and EDERSTROM, H. E. Relations between cutaneous blood flow and sweating at various environmental temperatures, *Am. J. Physiol.*, **163**:743, 1950.
 146. RANDALL, W. C., and McCLURE, W. Quantitation of the output of the sweat glands and their response to normal stimulation, *Am. J. Physiol.*, **155**:462, 1948.
 147. ———. Quantitation of the output of individual sweat glands and their response to stimulation, *J. Appl. Physiol.*, **2**:72-80, 1949.
 148. RANSON, S. W. The hypothalamus as a thermostat regulating body temperature, *Psychosom. Med.*, **1**:486-95, 1939.
 149. ———. Regulation of body temperature, *A. Res. Nerv. & Ment. Dis. Proc.*, **20**:342-99, 1940.
 150. RANSON, S. W.; FISHER, C.; and INGRAM, W. R. Hypothalamic regulation of temperature in the monkey, *Arch. Neurol. & Psychiat.*, **38**:445-66, 1937.
 151. RICHTER, W. Beiträge zur normalen und pathologischen Anatomie der apokrinen Hautdrüsen des Menschen mit besonderer Berücksichtigung des Achselhöhlenorgans, *Virchows Arch. f. path. Anat.*, **287**:277-96, 1932.
 152. RING, J. R., and RANDALL, W. C. The distribution and histological structure of sweat glands in the albino rat and their response to prolonged nervous stimulation, *Anat. Rec.*, **99**:7-19, 1947.
 153. ROTHMAN, S. Über die Wirkung des ultravioletten Lichtes auf die unmerkliche Wasserabgabe der Haut, *Strahlentherapie*, **35**:381-86, 1930.
 154. ———. Systemic disturbances in recalcitrant *Trichophyton rubrum* (purpureum) infections. Studies and short report on therapeutic experiments, *Arch. Dermat. & Syph.*, **67**:239-46, 1953.
 155. ROTHMAN, S., and COON, J. M. Pilomotor action of nicotine; a new pharmacodynamic test of the skin, *Arch. Dermat. & Syph.*, **40**:999-1000, 1939.
 156. ———. Axon reflex responses to acetylcholine in the skin, *J. Invest. Dermat.*, **3**:79-97, 1940.
 157. ROTHMAN, S., and SCHAAF, F. Chemie der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1/2**:161-377. Berlin: J. Springer, 1929.
 158. SAITO, K. On local sweating on the heated area of the skin in man, *J. Orient. Med.*, **13**:511, 1930. Quoted by KUNO (90).
 159. SAMBERGER, F. Chromidrosis, *Dermat. Wchnschr.*, **109**:806-12, 1939.
 160. SCHIEFFERDECKER, P. Die Hautdrüsen des Menschen und der Säugetiere, ihre biologische und rassenanatomische Bedeutung, sowie die Muscularis sexualis. Stuttgart: E. Schweizerbart, 1922.
 161. SCHILF, E., and MANDUR, J. Zur Frage der Hemmungsinervation der Schweissdrüsen, *Arch. f. d. ges. Physiol.*, **196**:345-57, 1922.
 162. SCHWARTZ, H. G. Reflex activity within the sympathetic nervous system, *Am. J. Physiol.*, **109**:593-604, 1934.
 163. ———. The effect of experimental lesions of the cortex on the "psychogalvanic reflex" in the cat, *Arch. Neurol. & Psychiat.*, **38**:308-20, 1937.
 164. SCOTT, J. C., and BAZETT, H. C. Temperature regulation, *Ann. Rev. Physiol.*, **3**:107-30, 1941.
 165. SEITZ, L. Über die sogenannte Achselhöhlenmilchdrüse und deren Genese; Schwangerschaftsmetamorphose der Schweissdrüsen, *Arch. f. Gynäk.*, **88**:94-131, 1909.
 166. SHELLEY, W. B. Apocrine sweat, *J. Invest. Dermat.*, **17**:255, 1951.
 167. SHELLEY, W. B.; HORVATH, P. N.; and

- PILLSBURY, D. M. Anhidrosis; an etiologic interpretation, *Medicine*, **29**:195-224, 1950.
168. SHELLEY, W. B., and HURLEY, H. J. Methods of exploring human apocrine sweat gland physiology, *Arch. Dermat. & Syph.*, **66**:156-61, 1952.
169. ———. Localized chromidrosis: a disorder of the apocrine gland, *J. Invest. Dermat.*, **19**:265-66, 1952.
170. ———. The physiology of the human axillary apocrine sweat gland, *ibid.*, **20**:285-97, 1953.
171. SHELLEY, W. B., and MESCON, H. Histochemical demonstrations of secretory activity in human eccrine sweat glands, *J. Invest. Dermat.*, **18**:289-301, 1952.
172. SONNENSCHN, R. R. Local sweating in man induced by intradermal epinephrine, *Proc. Soc. Exper. Biol. & Med.*, **71**:654-56, 1949.
173. SONNENSCHN, R. R.; KOBRIN, H.; and GROSSMAN, M. I. Further observations on local action of epinephrine on human sweat glands, *Am. J. Physiol.*, **159**:591-92, 1949.
174. SPERLING, F., and KOPPANYI, T. Histo-physiologic studies on sweating, *Am. J. Anat.*, **84**:335-63, 1949.
175. STEWART, W. B.; SNOWMAN, R. T.; YUILE, C. L.; and WHIPPLE, G. H. Radio iron excretion by the skin and kidney of dogs, *Proc. Soc. Exper. Biol. & Med.*, **73**:473-75, 1950.
176. STÖHR, P., JR. *Mikroskopische Anatomie des vegetativen Nervensystems*. Berlin: J. Springer, 1928.
177. STÖHR, P., JR., and LEWIS, F. T. (eds.). *A textbook of histology; arranged upon an embryological basis*. By J. L. BREMER; rewritten by H. L. WEATHERFORD. 6th ed. Philadelphia: Blakiston Co., 1944.
178. SULZBERGER, M. B.; HERRMANN, F.; KELLER, R.; and PISHA, B. V. Studies of sweating. III. Experimental factors influencing the function of sweat ducts; a preliminary report, *J. Invest. Dermat.*, **14**:91-119, 1950.
179. SULZBERGER, M. B.; ZAK, F. G.; and HERRMANN, F. Studies of sweating. II. On the mechanism of action of local antiperspirants, *Arch. Dermat. & Syph.*, **60**:404-18, 1949.
180. SZODORAY, L. Heterotopic apocrine glands, *Orvosi hetil.*, **4**:360, 1948. Quoted in *Excerpta med.*, Sec. XIII, **3**:382, 1949.
181. TAKAHARA, K. Variation of the insensible perspiration due to cooling and warming of the skin, *J. Orient. Med.*, 1934. Quoted by KUNO (90).
182. TANKEL, H. I. A case of gustatory sweating, *J. Neurol., Neurosurg. & Psychiat.*, **14**:129-33, 1951.
183. THAUER, R. Der Mechanismus der Wärmeregulation, *Ergebn. d. Physiol.*, **41**:607-805, 1939.
184. THURMON, F. M., and OTTENSTEIN, B. Studies on the chemistry of human perspiration with especial reference to its lactic acid content, *J. Invest. Dermat.*, **18**:333-39, 1952.
185. UNNA, P. G. Kritisches und Historisches über die Lehre von der Schweißsekretion, *Schmidts Jahrb. d. in- u. ausländischen ges. Med.*, **194**:89-99, 1882.
186. UPRUS, V.; GAYLOR, J. B.; and CARMICHAEL, E. A. Localized abnormal flushing and sweating on eating, *Brain*, **57**:443-53, 1934.
187. VALLANCE-OWEN, J. The histological demonstration of glycogen in necropsy material, *J. Path. & Bact.*, **60**:325-27, 1948.
188. WADA, M. Sudorific action of adrenalin on the human sweat glands and determination of their excitability, *Science*, **111**:376-77, 1950.
189. WADA, M.; ARAI, T.; TAKAGAKI, T.; and NAKAGAWA, T. Axon reflex mechanism in sweat responses to nicotine, acetylcholine, and sodium chloride, *J. Appl. Physiol.*, **4**:745-52, 1952.
190. WAEELSCH, L. Über Veränderungen der Achselschweißdrüsen während der Gravidität, *Arch. f. Dermat. u. Syph.*, **114**:139-60, 1913.
191. WAGENAAR, J. H. Some observations upon a pilomotor reaction in response to faradism, *Arch. néerl. de physiol.*, **16**:43-54, 1931.
192. WANG, G. H., and LU, T. W. Galvanic skin reflex induced in the cat by stimulation of the motor area of the cerebral cortex, *Chinese J. Physiol.*, **4**:303-26, 1930.
193. WANG, G. H., and RICHTER, C. P. Action currents from the pad of the cat's foot produced by stimulation of the tuber cinereum, *Chinese J. Physiol.*, **2**:279-83, 1928.
194. WAY, S. C., and MEMMESHEIMER, A. The sudoriparous glands. II. The apocrine glands, *Arch. Dermat. & Syph.*, **38**:373-82, 1938.

195. WEDDELL, G. The anatomy of cutaneous sensibility, *Brit. M. Bull.*, **3**:167-72, 1945.
196. WEINER, J. S. The regional distribution of sweating, *J. Physiol.*, **104**:32-40, 1945.
197. WHITMAN, E. N. Neurological aspects of body temperature. Diss., University of Chicago, 1951.
198. WILKINS, R. W.; NEWMAN, H. W.; and DOUPE, J. The local sweat response to faradic stimulation, *Brain*, **61**:290-97, 1938.
199. WILSON, W. C. Some aspects of sweat secretion in man, with special reference to the action of pilocarpine, *Brain*, **57**:422-42, 1934.
200. WOOLLARD, H. H. The cutaneous glands of man, *J. Anat.*, **64**:415-21, 1930.
201. YAMADA, G. Quantitative investigations on the accessory apparatus of the skin in a German, *Keio-Igaku*, **11**:2709, 1931. Quoted by KUNO (90).
202. YUYUAMA, H. Über die histologische Untersuchung der Glykogenverteilung in der leprösen Haut, mit besonderer Berücksichtigung der Beziehung zwischen der Funktion der Schweissdrüsen und der Schwankung des Glykogens, *Jap. J. Dermat. & Urol. (abstr. sec.)*, **37**:134-36, 1935.

Addendum.—For some additional recent references see W. C. Randall, Special review. The physiology of sweating, *Am. J. Phys. Med.* **32**:292-318, 1953.

CHAPTER 7

Composition of Eccrine Sweat and Aqueous Surface Film

I. GENERAL	201
II. NITROGENOUS COMPOUNDS	202
A. Total Nitrogen Loss	202
B. Urea	203
C. Creatinine and Creatine	205
D. Uric Acid	205
E. Amino Acids	207
F. Ammonia	208
G. Choline	209
III. GLUCOSE	209
IV. LACTIC ACID	210
V. WATER-SOLUBLE VITAMINS	211
A. Vitamin B	211
B. Vitamin C	212
VI. ELECTROLYTES	212
A. Chloride and Sodium on the Dry Skin	212
B. Chloride and Sodium Content of Eccrine Sweat	213
C. Dermal Loss of Potassium	215
D. Dermal Loss of Other Minerals	215
1. Calcium	215
2. Magnesium	216
3. Sulfates and Phosphates	216
4. Iron	216

I. GENERAL

ECCRINE sweat is a clear aqueous solution which contains 99.0–99.5 per cent water and 0.5–1 per cent solids. Half the solids are inorganic salts, and half consist of organic substances. Most of the inorganic ash is sodium chloride, and half the organic material is urea. The specific gravity of profusely secreted sweat is normally between 1.001 and 1.006. It was claimed that sweat obtained in strenuous exercise is more concentrated than thermal sweat obtained at rest (80). Intermittently secreted palmar sweat

must have an unusually high specific gravity because of the high concentrations of salts and organic materials found in such sweat (44).

Under normal circumstances eccrine sweat is hypotonic (23, 39). At high rates of sweating it may approach almost isotonic concentration (39). Its average osmomolecular concentration corresponds with a freezing point of $-0^{\circ}32$ C. (80), as contrasted with $-0^{\circ}56$ C. in the blood. Ninety-five per cent of the osmotic pressure of sweat is due to

chlorides, lactic acid, and urea (104). Sodium chloride accounts for 80 per cent, and lactate for 11 per cent (104).

Comparison of the chemical composition of blood plasma and that of sweat shows that, although the composition of sweat depends, to some extent, on the material offered by the blood stream, sweat is not a simple ultrafiltrate of blood plasma but the product of an active secretory function. It resembles plasma ultrafiltrate in its general inorganic pattern. Like plasma, it has large amounts of sodium and chloride, but little potassium and phosphate; concentrations of potassium and calcium are about the same as in blood serum (18). The ratio of sodium to chloride is of the same order of magnitude in the two fluids (18). However, the quantitative differences make it impossible to interpret sweat formation as a simple ultrafiltration process. The concentration of sodium and chloride is always lower, that of potassium sometimes higher, in sweat than in plasma (66, 93, 18). Urea is secreted by the sweat glands in a concentration twice as high as that of blood. The total nitrogen content may be also much higher than the nonprotein nitrogen in plasma (34). Sweat glands are rather efficient barriers against the elimination of glucose. All this, of course, is reminiscent of the kidney function. But the old idea of the vicarious function of sweat glands in kidney insufficiency certainly cannot be maintained. Even if sweat glands

have the ability to concentrate urea, "the most active skin is less efficient for excretion of nitrogenous products than the most seriously injured kidney" (59). It is rather impressive if, premortally, in uremia and in cholera the urea concentration of sweat is so high that it forms visible crystals on the skin surface after the water has evaporated. But even the most profuse single sweating bout will yield less than 1 gm. of nitrogen (80), and in uremia the sweat mechanism is suppressed anyhow, probably in consequence of simultaneous salt retention.

Physiologically, the kidneys play a vital role in regulating the composition of blood plasma, in the water, salt, and acid-base equilibrium, and in removing metabolic end-products. Sweat glands do not perform such functions to any significant degree (23, 15).

The constituents of sweat are present also on the apparently dry skin surface. The surface film of the skin probably represents a fine emulsion of an aqueous and a lipid phase, and the water-soluble sweat constituents may be present in the aqueous phase of this emulsion. In physiological work the interest has been centered on the repercussions of the loss of water and salt as it occurs in profuse sweating under extreme conditions. From the dermatological point of view, the composition of the film on the nonsweating skin surface is the most important topic.

II. NITROGENOUS COMPOUNDS

A. TOTAL NITROGEN LOSS

Under comfortable conditions without visible sweating, the dermal loss of nitrogen in the adult was estimated to be 0.1–0.3 gm. daily (66, 59). It was emphasized that there is measurable dermal loss of nitrogen even if no sensible perspiration can be detected (7). Of course, the old difficulty in separating nitrogen losses caused by sweat-gland activity and those by epidermal desquamation (66) is still not solved, in spite of the classical experiments of E. Voit (103). His experiments, carried out on a twenty-

eight-year-old healthy man, yielded an average daily dermal loss of 332.8 mg. of nitrogen. Out of this amount, 121.9 mg. was keratin-N (corresponding to 776.5 mg. keratin), and 210.9 mg. was "extract-N," this fraction including all water- and alcohol-soluble nitrogenous compounds. Thomas (96) obtained 130 mg. nitrogen per day by rinsing his clothes with water. The "extract-N" of Voit could originate from two sources: from insensible sweat secretion and from water-soluble nitrogenous products of the keratinization process, such as amino acids

and uric acid (5), which are shed with the horny lamellae (see chap. 16).

There can be little doubt that sweat-gland function goes on continuously even at low temperature (p. 156); and obviously nitrogenous material is secreted with the insensible sweat as well as with visible sweat. One may assume that the major part of the water-soluble nitrogen loss is due to sweat-gland activity. It was shown, for instance, that in the absence of sweat glands, as is the case in anidrotic ectodermal dysplasia, amino acid excretion to the surface is almost impossible to demonstrate (68). From the values of Voit (103) one can conclude that at rest, without visible sweating, roughly a third of the nitrogen loss is caused by epidermal shedding and two-thirds by insensible sweat secretion. The average total dermal loss of nitrogen per surface area (103) was calculated to be 136.4 mg/sq meter/day in Voit's nonsweating (103) resting subject. Out of this amount, 50.0 mg. nitrogen were due to loss with horny lamellae. This corresponds to the shedding of 318.2 mg of keratin/sq meter/day.

By following the rate of growth of hair and nails over periods of many years in several individuals, Voit (101, 102) estimated the nitrogen losses deriving from the formation of these appendages. The weight of hair grown in one year varied between 56.15 and 63.01 gm. in two younger individuals and was 41.70 gm. in an older person. The weight of fingernails and toenails grown in one year averaged a total of 2 gm. All this corresponds roughly with a daily loss of 24 mg. of nitrogen originating from hair and nail growth.

Voit (103) gives an account of losses of keratinized material "per square meter of surface and per year in grams" in a twenty-eight-year-old man (see accompanying tabulation).

Scalp hair.....	11.4
Facial hair.....	9.8
Body hair.....	8.3
Finger- and toenails.....	(2.02)
Desquamating epidermis.....	116.14
Total.....	147.66

The amount of nitrogen lost with the sweat increases rapidly with increasing environmental temperature or with exercise. The secretion of nitrogen in sweat is roughly proportional to the amount of exercise (7). But, as sweat becomes more profuse, its nitrogen concentration, as contrasted with the increasing concentration of electrolytes, decreases (15) (cf. Table 3 on p. 215). In the experiments of Moore *et al.* (56) the nitrogen secretion with sweat varied between 14.7 and 99.4 mg/sq meter/hr. They found food intake without any influence on these values.

B. UREA

The urea concentration of sweat is always higher than that of the blood. The quotient of sweat-urea-N/plasma-urea-N is 2:1 or greater. For a review of the literature on this remarkable phenomenon see Rothman and Schaaf (66) and Lobitz and Osterberg (48). In the older literature the concentration in profuse thermoregulatory eccrine sweat is given as varying between 23.5 and 400 mg. urea (!) in 100 cc. of sweat, corresponding with 11–186 mg/100 cc urea-N. The most commonly encountered values are given as being between 30 and 60 mg. urea-N in 100 cc. The average concentration of urea-N in blood plasma is 12–15 mg. Gad-Andresen (19) found an average value of 44.9 mg/100 cc urea-N in sweat, as contrasted with 22.9 mg/100 cc in the blood.

Lobitz (48, 44) studied in detail the urea content of uncontaminated palmar sweat and found an amazing difference in the urea content of profusely and intermittently secreted sweat samples, the average values being, for the first type, 68 mg/100 cc and, for the second, 275 mg/100 cc urea (Fig. 1). These differences could be demonstrated even in the same persons in whom it was possible to obtain sweat samples of the two different types in rapid succession under otherwise identical experimental conditions.

On feeding or injecting urea, the urea concentration of sweat rises and falls with that of the blood; but during the whole period the sweat values are higher than those

in blood (Fig. 1). The urea concentration in the urine, however, is still higher (48). There is no detectable correlation between urinary and sweat urea.

The mechanism whereby the sweat glands manufacture a solution with a much higher urea content than is offered to them with the blood is not clear. It could be selective uptake, sometimes designated as "active storage"; it could be a result of reab-

sorption of water from the ducts (p. 190); or it could be new formation of urea in sweat-gland cells. The latter possibility was first considered by Hier *et al.* (26), who found large amounts of arginine in sweat and suggested that possibly urea is manufactured in the sweat glands by the enzyme arginase, which splits arginine into ornithine and urea. That this actually might be the case was indicated by Rothman and Sullivan (68) when in water-wipings of the dry skin they tentatively identified ornithine as one of the amino acids on the skin surface. In

sweat itself no arginase could be demonstrated (99). This, of course, does not exclude the presence of arginase within the secretory cells, but so far the new formation of urea in sweat glands remains to be demonstrated. The problem can probably be decided by the use of radioactive arginine.

In a recent contribution Schwartz *et al.* (79) found a constant linear relationship between sweat and plasma urea concentrations, with a ratio of sweat urea to plasma urea of 1.752. This ratio was found to be independent of the plasma urea concentration in the range of from 12 to 300 mg. per cent and independent of the rate of sweat secretion. The authors interpreted their experimental results as indicating that about half the water secreted by the glands is reabsorbed over a wide range of flow. They deemed it unnecessary to assume a special mechanism for selective urea pickup (or production of urea) by the glands. A special pickup mechanism seemed to be improbable, in view of the huge energy requirements involved.

The urea content of apocrine sweat has not been investigated. But it appears that apocrine glands are avid secretors of urea if its concentration in the blood is high. Scheunert *et al.* (74) fed urea to sheep in amounts which caused the blood urea concentration to increase up to fivefold. During the periods of urea feeding, the washings of the total skin surface yielded 1 gm. nitrogen daily (excluding keratin-N), whereas in periods when a diet rich in proteins, but with no urea, was fed, the corresponding amount was only 0.013 gm. nitrogen. In other words, when the blood level was raised fivefold, the skin excretion increased almost eighty fold. In periods of low protein diet, only traces of nitrogen could be detected in the washings. Sheep have only apocrine glands on their trunks. If the excretion of nitrogen through the skin after the feeding of urea is actually a sweat-gland function, as Scheunert *et al.* (74) assume, it is indeed remarkable that this apocrine function takes place without any profuse sweat secretion. Moore, Peters, *et al.* (56) could not demonstrate such a

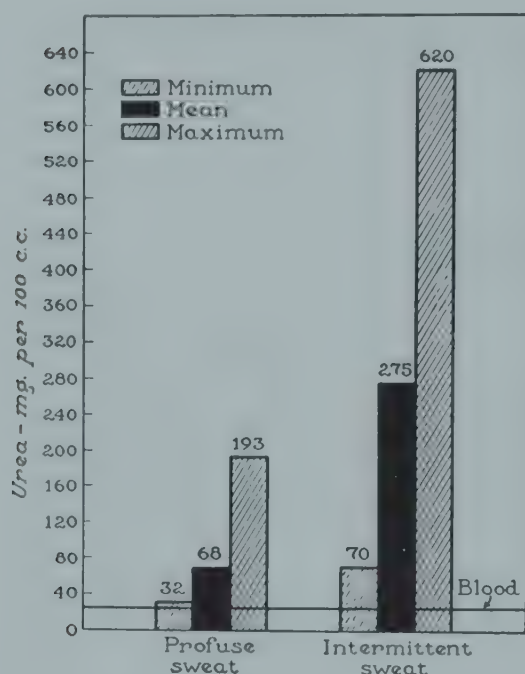


FIG. 1.—The mean value of urea (expressed as milligrams urea per 100 cc. of sweat), with minimum and maximum for the two physiological types of sweating and mean value for blood urea in all types. From Lobitz and Osterberg (48). (Reproduced by permission of Dr. Lobitz and the American Medical Association.)

sorption of water from the ducts (p. 190); or it could be new formation of urea in sweat-gland cells. The latter possibility was first considered by Hier *et al.* (26), who found large amounts of arginine in sweat and suggested that possibly urea is manufactured in the sweat glands by the enzyme arginase, which splits arginine into ornithine and urea. That this actually might be the case was indicated by Rothman and Sullivan (68) when in water-wipings of the dry skin they tentatively identified ornithine as one of the amino acids on the skin surface. In

function of human eccrine glands. They administered large doses of urea to normal persons but did not find any increase in the sweat nitrogen after such administration, in spite of hot weather.

Urea is deposited in substantial amounts on the normal skin surface in man also, without any visible sweat secretion. This was demonstrated by Rothman and Smiljanic (67), who found that urea-N constitutes one-fourth to one-third the total nitrogen found in water-wipings of the normal adult skin surface under comfortable conditions with no visible sweating.

After visible sweating the absolute amount of urea which is deposited on the skin will obviously increase. Changes in the urea content of the skin surface (individual differences and differences in the same person under various physiological and pathological conditions) might have considerable significance in dermatology. For studies in this field the amount of urea probably will have to be related to units of skin surface area.

C. CREATININE AND CREATINE

Creatinine is present in sweat in minute quantities, and its presence cannot always be demonstrated (66, 44). In most cases its concentration was found to be less than 2 mg/100 cc. In heat sweat of young healthy adults Schumann (78) found sweat values mostly between 3.5 and 5 mg/100 cc, but less when sweating was more profuse. In single patients with different internal diseases, values as high as 6.68 mg/100 cc were registered. There was no relation to the level of blood creatinine. In some samples creatine could also be demonstrated. Schumann thought that the acidity of sweat may convert creatine into creatinine and that the pH of the sweat sample may determine whether or not creatine can be found.

The observation that the slower the sweating rate, the higher the creatinine concentration was duplicated by Ladell (40). This behavior is in contrast to that of chlorides, the concentration of which increases with increasing rate of sweating (p. 213). However, the concentration values of Ladell

(40) are considerably lower than those of Schumann. In sweat elicited by heat and by exercise Ladell found initial values in the neighborhood of 1 mg. in 100 cc., and a considerable drop, to 0.3 mg. in 100 cc. or even less, when sweating continued for 1 hour. The final values, after sweating had gone on for 1½ hours, were somewhat higher again (Fig. 2). The assumption that the higher initial values might have been due to creatinine or other substances with the same chemical reaction deposited on the dry skin, which would be washed off when sweating sets in, could not be substantiated (40).

Analyses in my laboratory showed that creatinine-N is present in small amounts on the dry skin surface. Creatinine-N constitutes 1–2 per cent of the total nitrogen in water-wipings of normal nonsweating human skin surface (67). On intake of creatinine, the increase in concentration in profuse thermoregulatory sweat (40) and in profuse palmar sweat (44) is, as compared with the increase in blood plasma and in saliva, relatively small. This is in contrast to the behavior of the kidneys. Urinary output of creatinine is greatly increased by creatinine intake, but the output in sweat is not.

D. URIC ACID

After many contradictory data have been published on the uric acid content of eccrine sweat (66), there is general agreement today that the eccrine glands in man are poor secretors of uric acid. Its concentration in thermogenic sweat is always lower than in the blood, varying between 0 and 1.7 mg. in 100 cc. of sweat (43). Saiki and Talbert's (70) maximum value had been 0.25 mg. per cent. Lobitz and Mason (43) found no measurable amounts in profuse palmar sweat and an average of 0.8 mg/100 cc in intermittent palmar sweat. When lithium urate was given intravenously, there was a very moderate increase of uric acid concentration in sweat, not comparable to that in the urine. However, Williams (107) states that he was able to drive the concentration up to 30 mg. per cent by a diet rich in nucleoproteins.

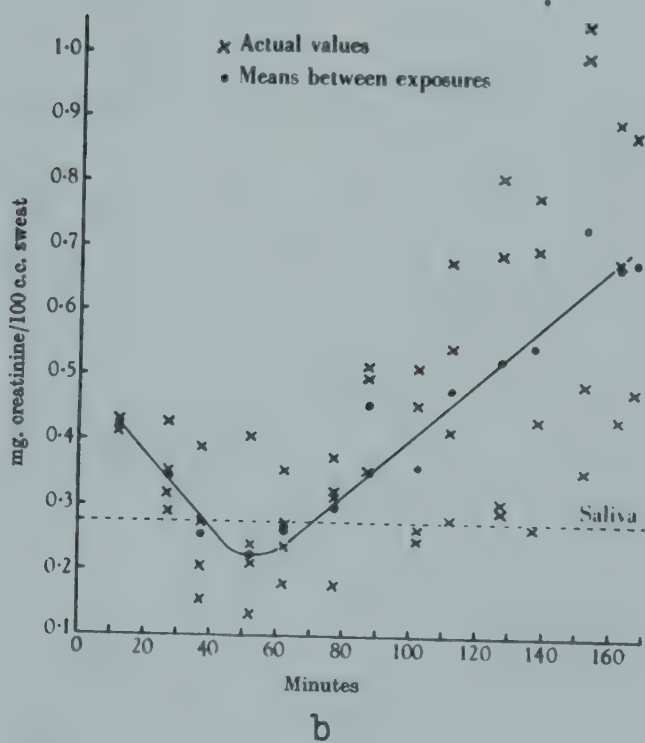
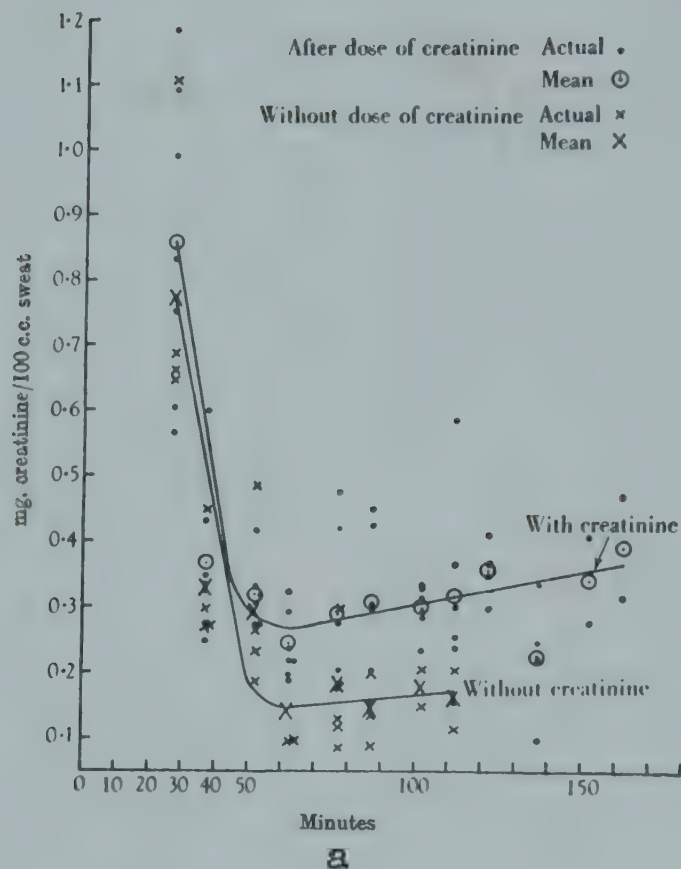


FIG. 2.—Changes in creatinine concentration in the sweat during exposure to heat. *a*, subject *G*: with and without a dose of creatinine before the test. *b*, subject *L*: without creatinine. The dotted line indicates the concentration of creatinine in the saliva (mean of three observations). From Ladell (40). (Reproduced by permission of the Journal of Physiology.)

On the dry nonsweating normal skin uric acid is present only in traces. In water-wipings from a surface approximately 2 square meters in area, no measurable amounts could be recovered (67).

E. AMINO ACIDS

The presence of amino acids in thermogenic sweat has been known since 1910, when Embden and Tachau (17) isolated serine from human sweat. According to

Sullivan (68) demonstrated the following amino acids by paper chromatography: aspartic, glutamic, serine, glycine, alanine, citrulline, arginine, proline, hydroxyproline, lysine, valine, one of the leucines, and tyrosine. Tentatively identified were hydroxylysine, threonine, lysine, and ornithine (Fig. 3). The identification of arginine and citrulline (a postulated intermediary in the arginine cycle of Krebs) (Fig. 4) and the tentative identification of ornithine sup-

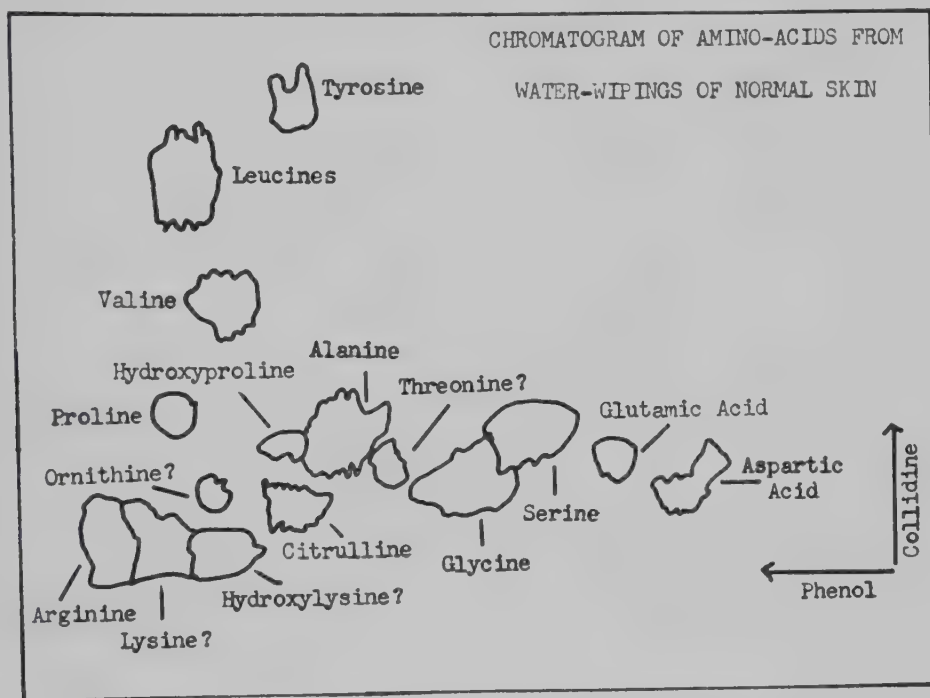


FIG. 3.—Amino acids on the normal human skin surface. Modified from Rothman and Sullivan (68). (Reproduced by permission of the Williams & Wilkins Company.)

Talbert *et al.* (24), the concentration of amino-N in sweat is between 1.57 and 4.76 mg/100 cc. A detailed analysis by microbiological methods was done by Hier, Cornbleet, and Bergheim (26), who identified the following amino acids in sweat, listed in order of decreasing concentration: arginine, histidine, threonine, tyrosine, valine, leucine, isoleucine, lysine, phenylalanine, and tryptophane.

Rothman, Smiljanic, and Murphy (67) found that more than half the nitrogenous material on normal nonsweating human skin surface is amino nitrogen. In water-wipings from such surfaces, Rothman and

ported the assumption that urea is formed in the sweat glands from arginine (p. 204). It should be pointed out that the amino acids on the skin surface might be of clinical significance as a feature of its "chemical milieu." Individual and regional differences in the composition of this milieu might be responsible for differences in susceptibility and resistance to infections and infestations and also to any disease which may have a "constitutional" background.

It would be of importance to establish whether amines, such as asparagine and glutamine, deriving from the respective amino acid by decarboxylation, do occur generally,

individually, or regionally on the skin surface, for these compounds are excellent nutrients of hyphomycetes and other microorganisms.

Rothman and Sullivan (68) tested the water-wipings of a patient with the anidrotic type of ectodermal dysplasia and found faint traces of serine and alanine only once. This would indicate that the main source of amino acids on the skin surface is the sweat glands. Still, on the basis of the work of Bolliger (5), one has to assume that amino acids on the skin surface are also by-

freshly secreted heat sweat, when secondary decomposition was carefully avoided, Gad-Andresen (19) reported a strict parallelism between blood and sweat ammonia-N and postulated a pure diffusion process. Talbert *et al.* (91), apparently working with not quite freshly secreted sweat, found no such correlation between blood and sweat ammonia. Secondary decomposition explains the widely divergent data of the literature on the ammonia content of thermogenic sweat, which varies from 2.5 to 35 mg. ammonia-N in 100 cc. (42, 91, 57).

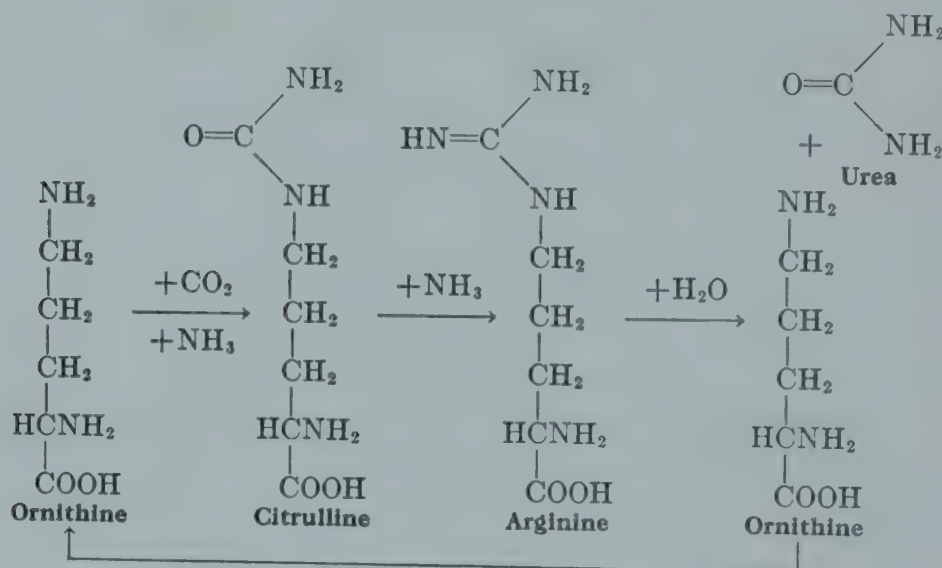


FIG. 4.—The Krebs arginine cycle

products of the keratinization process (p. 372).

Vermeer and DeJong (100) presented evidence that amino acids play a prominent part in the ability of the skin surface to neutralize fixed alkalis and acids (p. 228).

F. AMMONIA

Although there can be little doubt that ammonia is present in freshly secreted sweat, its amount certainly increases by bacterial decomposition of urea on the surface (66, 52). This decomposition is accompanied by an increase in the pH of the non-evaporating sweat. Talbert, Finkle, and Katsuki (91) reported that concentrations of ammonia-N and of urea-N in thermogenic sweat parallel each other to some degree. In

Lobitz and Mason (49) found striking differences in the ammonia content of profusely and intermittently secreted palmar sweat, the average being 7.7 and 28.9 mg. ammonia-N in 100 cc. in the two different types of sweat, respectively. This difference was obtained even in the same person according to his type of sweating and was independent of the order in which the different types of sweating were elicited. The authors concluded that ammonia not only is a primary constituent of sweat but can be concentrated by the sweat glands with nearly the same efficiency as by the renal excreting unit. Still the possibility remains that, in intermittent sweating, ammonia is formed in the ducts when the sweat stagnates there. In water-wipings of normal dry nonsweating

skin 3–6.5 per cent of the total nitrogen was found to be ammonia-N (67).

Ottenstein (58) has recently demonstrated unusually high ammonia-N values in axillary sweat, up to 78 mg. in 100 cc. (!), and she related this finding to the relatively low pH values obtained from sweat of this region. It may well be that these values are caused by secondary decomposition of urea, because Ottenstein's high ammonia-N values are associated with conspicuously low urea-N values.

G. CHOLINE

Free choline is present on the skin surface. In part, it is a product of phospholipid degradation as it occurs during the keratinization process (p. 489). How far sweat-gland activity may contribute to its presence on the surface was investigated by Johnson, Hamilton, and Mitchell (29). They fed choline to human subjects and studied its excretion in urine, feces, and through the skin under comfortable and under hot, humid conditions. Most of the choline given orally was metabolized, and only 0.7–1.5 per cent of the administered amount was excreted as choline. Of the excreted choline, 63.5 per cent was found in the urine, 36.2

per cent in feces, and only 0.3 per cent was excreted through the skin in nonsweating individuals. During the hot, moist period the respective figures were 55 per cent in urine, 43.6 per cent in feces, and 0.9 per cent in sweat. Thus choline can apparently be secreted by sweat, even if only in small amounts. The concentration of choline in undiluted sweat in these experiments was 2.7–15.3 γ /1,000 cc. Sweating did not bring about an absolute increase in total choline excretion.

In the past there has been much discussion of choline as a menstrual toxin excreted through the skin and as the cause for the alleged wilting of flowers in the hands of a menstruating woman (66). It was claimed that the dermal choline excretion is increased twenty to sixty fold during menstruation (82). The choline content of the blood actually fluctuates with the menstrual cycle, but the fluctuation parallels that of the estrogenic hormone content of the blood. Its peak concentration is reached on the fourteenth day of the intermenstruum, and the minimum concentration is found around the twenty-sixth day (75). Therefore, it is unlikely that there is an increased excretion of choline during menstruation.

III. GLUCOSE

The literature on the occurrence of glucose and other reducing substances in sweat was reviewed by Lobitz and Osterberg (47). They pointed out that the contradictory results might be explained by the different methods of collecting and handling sweat and by differences in methods used for estimating the reduction. Certainly, the concentration of reducing substances in sweat is much lower than data in the earlier literature indicated (66) and very much lower than in the blood.

Lobitz and Osterberg (47), examining palmar sweat, found a mean value of 3.08 mg. in 100 cc. (range from 0 to 11 mg.) in profuse sweat and a mean value of 2.86 mg. in 100 cc. (range from 0 to 16 mg.) in intermittent sweat. This is contrasted with a

mean blood sugar value of 86 mg. in 100 cc. (range from 74 to 100 mg.). All this indicates that the sweat gland is an efficient barrier against the elimination of sugar, even though not such a perfect barrier as the kidneys are. Whether this is simply an effect of impermeability is not known. Glucose offered by the blood stream could possibly be utilized by sweat-gland cells for the synthesis of glycogen, or it could be metabolized to lactate or aerobically to carbon dioxide and water.

In contrast to Marchionini and Ottenstein (53), most authors do not find any correlations between the sugar content of blood and of sweat (47, 83) either in diabetic hyperglycemia or after oral and intravenous administration of glucose. The results of

Lobitz and Osterberg (47), however, were not uniformly negative. In one case after intravenous injection of glucose they obtained 18 mg. glucose in 100 cc. of sweat, which is considerably higher than their average value.

The whole situation is rather complicated because all investigators calculated the total

TABLE 1* (76)

SUM OF REDUCING SUBSTANCES EXPRESSED
AS GLUCOSE IN MG/100 SQ CM

Subjects	1st Removal	2d Removal	3d Removal
Nondiabetic.....	0.070	0.036	0.021
Diabetic.....	0.086	0.033	0.028

* Warm water applied to abdominal skin under a glass bell 8.5 cm. in diameter for 10 minutes, and subsequently twice more for 10 minutes in each experiment. The values are averages of 10 experiments in each normal and in the diabetic person.

sum of reducing substances as if it were glucose. Yet there is evidence that glucose is only a fraction of the total reducing material. Schulze (76, 77) presented evidence that this fraction is surprisingly small. A further complication arises from the presence of reducing sugars on the skin surface, deriving from the disintegration of keratinizing cells (5, 98) (p. 374).

Concerning the presence of glucose on the nonsweating skin surface, Marchionini and Ottenstein (53), as well as Carrié and

Koenig (10), found by the so-called "dialysis method" (application of water to the skin under a glass bell) measurable amounts of reducing substances in normals and increased amounts in diabetics. These experiments were criticized by Schulze (76), who was unable to confirm the findings of increased amounts in diabetics. The experiments of Schulze himself, however, indicate that there is a deposition of reducing substances on the dry skin surface. When he made repeated applications of warm water to the same skin area under a glass bell in rapid succession and estimated the amount of reducing substances in the subsequent "dialysates," the first sample proved to be considerably higher in reducing materials than did the subsequent ones (Table 1).

In order to find the true glucose content in sweat and on the skin surface, Schulze and Kunz (77) used the quantitative method of estimating the phenylglucosazon derivative of glucose. In this way they found that only about one-tenth of the sum of reducing substances represents glucose. What the rest is, would be rather important to know. Schulze and Kunz indicated that it could be some phenolic substances, as they found 2-8 mg. phenols in 100 cc. of heat sweat. But they also considered the possibility that reducing substances are being formed during the procedure when the blood sugar estimation method of Hagedorn-Jensen is applied to sweat.

IV. LACTIC ACID

Since the basic observations of Schenk in 1926 (73) and of Snapper in 1928 (84) on athletes, the presence of large amounts of lactic acid and lactate in sweat has often been confirmed. During heavy muscular exercise for $1\frac{1}{2}$ hours, as much as 2.2 gm. may be secreted with the sweat. Similarly, large amounts are secreted with thermogenic sweat (see Table 2).¹ The concentrations found are ten to twenty times higher than in the blood, and it has been assumed that the excess lactic acid originates from the break-

down of glycogen in the sweat glands (104). It appears that lactic acid in sweat derives from some anaerobic glycolytic process, because in the sweat of an extremity the venous circulation of which is occluded, the lactic acid concentration is considerably increased. Dill *et al.* (15) doubt that in thermogenic sweating, with maximal blood supply to the glands, anaerobic glycolysis would prevail; but direct investigation of this possibility is lacking.

Fishberg and Bierman (18) state that about half the lactic acid is present as free

¹ For earlier data see Rothman and Schaaf (66).

acid in sweat, and it often has been assumed that this free lactic acid contributes to the acidity of sweat and skin surface (65, 97). Also it has been suggested that the lactic acid/lactate buffer system contributes to the buffering capacity of the skin surface (58).

Elimination of that much acid with the sweat should decrease the loss of base (18, 34). However, Dill *et al.* (15), from calculations of the acid-base balance of sweat, do not believe that lactic acid secretion in acclimatized persons has such an effect.

The lactic acid content of sweat was found by Thurmon and Ottenstein (97) to be higher in men than in women, a difference which measurably influenced the pH, causing more acid sweat in men (chap. 8). There is no difference in the lactic acid concentration of axillary and body sweat, but it is consistently higher in the sweat of males than in that of females (58). It increases with environmental thermal severity under both dry and humid conditions (104). The most remarkable finding in this field has been that in the process of acclimatization the lactate concentration, like that of sodium chloride, decreases considerably. After 10–15 daily 3-hour exposures, it may fall from 300 to 100 mg. per cent (104, 105). Dill's values (15) in acclimatized individuals are as low as 45–76.5 mg. in 100 cc. (Table 2). Whereas the decrease in sodium chloride concentration has been satisfactorily ex-

plained as a pituitary-adrenal response to stress (p. 265), the decrease in lactic acid remains a baffling problem.

In seborrheic dermatitis, increased amounts of lactic acid were found in sweat on the scalp, as compared with normals, and itching was attributed to this excess (4). In

TABLE 2
LACTIC ACID CONCENTRATION
IN THERMOGENIC SWEAT

Author	Date	Concentration (Mg/100 Cc)	Remarks
Saiki, Olmanson, and Talbert (69)	1932	73–160	In acclimatized persons
Fishberg and Bier- man (18)	1932	250–350	
Weiner and van Hey- ningen (105)	1951	Up to 300	
Thurmon and Otten- stein (97)	1952	247–452	
Dill <i>et al.</i> (15)	1938	45–76.5*	

* Calculated from milliequivalent (mEq.) values.

acne patients, too, values higher than normal were reported, together with a higher acidity of the sweat (58). Unfortunately, in this work the two groups (acne patients and controls) represent two different age groups, and therefore no conclusions can be drawn about the role of acne.

V. WATER-SOLUBLE VITAMINS

A. VITAMIN B

The literature on the presence of water-soluble vitamins in thermogenic sweat up to 1944 was reviewed by Sargent, Robinson, and Johnson (71), and not much has been added since. In contrast to conclusions drawn from the earlier literature, these authors concluded that loss with sweat is not a significant factor in depleting the body of its water-soluble vitamins. They found that, even with daily losses of 10–15 liters of sweat, the excretion of vitamins, with a few exceptions, is greater in the urine than in

sweat. They emphasize that nobody ever worried about loss of vitamins in the urine and that there is even less reason to worry about sudoriferous losses.

The presence of the following B factors in thermogenic sweat has been demonstrated and, when possible, quantitatively estimated: niacin, the main metabolic excretion product being *N'* methyl-nicotinamide (30); pyridoxine, the main metabolic excretion product being 4-pyridoxic acid (28); folic acid (31); pantothenic acid (86, 71); biotin (32); inositol (32); and *p*-aminobenzoic acid

(32). Thiamine and riboflavin are either completely absent or present in infinitesimally small amounts; for instance, thiamine is certainly less than 0.5 γ /100 cc. It was shown that neither thiamine nor riboflavin is destroyed if added to freshly secreted sweat. Thus it can be concluded that they are actually not secreted by sweat glands (71).

A few remarkable features were noted: (1) excretion of folic acid in continuously profuse sweat is five to six times greater than with the urine (31) if the excretion is calculated on an hourly basis; (2) the dermal loss of inositol per hour increases about four-fold under hot, moist conditions, and at the same time its urinary excretion drops to lower levels (32); (3) if pyridoxine is administered, its excretion by sweat increases (28); (4) absolute amounts of pantothenic acid increase from 5.1 γ /hr under comfortable conditions to 27.7 γ /hr in a hot, moist atmosphere. But its concentration is higher in the insensible sweat under comfortable conditions than in actual sweat. This is

reminiscent of the higher concentration of other sweat constituents in slowly and intermittently secreted sweat, as established by Lobitz *et al.* (44). Remarkably, urinary excretion of pantothenic acid also increases in a hot, moist atmosphere. This behavior of pantothenic acid is in contrast to that of inositol.

B. VITAMIN C

Losses of ascorbic acid with the sweat were found to be negligible (71, 54) even under the extreme condition of losing 10 liters of sweat at one time. Ascorbic acid itself is not found in sweat, only its oxidation product, dehydroascorbic acid; its concentration does not exceed 0.2 mg. in 100 cc. sweat. Sweat oxidizes added ascorbic acid to the dehydro derivative. This is not an enzymatic process; the oxidizing principle is heat-stable. If sweat is boiled, the destruction of ascorbic acid is even accelerated. This might be due to liberation of some previously bound heavy-metal trace or to decomposition of protecting proteins (71).

VI. ELECTROLYTES

A. CHLORIDE AND SODIUM ON THE DRY SKIN

Under comfortable environmental conditions, the aqueous film of the "dry" skin surface (including the surfaces of the lamellae in the stratum disjunctum of the horny layer) always contains salt, because of the continuous "insensible" sweat secretion and the cell disintegration connected with keratinization and sebum formation.

If one endeavors to investigate the possible clinical significance of variations in the salt content of the surface, the values will have to be expressed as related to surface rather than in terms of concentration. Such work was initiated by Arn and Reimer (3). They measured the minimum dermal sodium losses in two normal male adults at sodium balance without visible activity of sweat glands. The total loss was found to be 4.06 and 4.40 mEq. Na (93.4 and 103.2 mg., respectively) per 24 hours. Calculated for

surface, this was 2.22 and 2.18 mEq. (or 51.06 and 50.14 mg.) Na per square meter in 24 hours. If all this sodium were attached to chloride, the amount of sodium chloride deposited on 1 square meter of the adult human male's skin surface would be an average of 128.7 mg. Obviously, this figure is modified by dietary intake, by exercise, and by sensory (mental) stimulation of sweat glands.

Hancock *et al.* (23) recovered 83 mg. chloride in the bathing water of an individual who had not bathed and not sweat visibly for 1 week. This would correspond to 136 mg. of sodium chloride over the whole body surface (excluding the head) if all chloride were attached to sodium. The authors believed that all this material originated from "sebaceous and keratinous" debris, because the bathing water contained almost as much potassium (77 mg.) as chloride. In fluid sweat the relation Cl:K is known to be

about 9:1 (p. 215). Schwenkenbecher (81) estimated the daily sodium chloride losses through nonsweating skin of a normal adult to be much higher, namely, 330 mg/day, on the average. He attributed this dermal loss of sodium chloride entirely to invisible sweat secretion. Bathing in water momentarily removes all chloride from the surface, but the replenishment is fast, as was shown by Carrié. His values were obtained by placing a water-filled glass cylinder on the skin and taking aliquot fractions of the fluid for analysis at regular intervals (9).

That the salt content of the horny layer is not necessarily caused by sweat imbibition is concluded from the fact that not only the ash of the horny layer but also that of hair always contains salt, even in parts far away from the surface. The participation of insensible sweat-gland activity in the deposition of salts on the skin surface could be investigated best by carrying out estimations before and after atropinization or by comparing the values in normals with those of patients with anidrotic ectodermal dysplasia.

B. CHLORIDE AND SODIUM CONTENT OF ECCRINE SWEAT

The concentration of chlorides in eccrine sweat depends on many factors and is extremely variable but is always hypotonic as compared with blood plasma (39). Calculated as sodium chloride, values from 0.06 to 0.5 per cent were reported in the older literature (66). Measurements of Ladell (39) in the Arabian desert yielded concentrations of from 0.1 to 0.6 per cent, with a mean of 0.29 per cent. Conn (11) found a mean of 0.4 per cent in unacclimatized individuals. Kittsteiner (36, 37), E. F. Adolph (2), and Hancock *et al.* (23) claimed that 0.5 per cent sodium chloride concentration is a maximum never surpassed. Ladell (39) himself found that isotonicity with blood plasma is approached only under extremely severe conditions. Sodium concentration is almost entirely equivalent with that of chloride, and they vary in a parallel fashion. The normal range of sodium concentration is

given as 0.03–0.138 per cent or 15–60 mEq/l (61).

The first factor influencing chloride concentration is the duration of thermal sweating during a single exposure: the chloride concentration rises with time, and this continues independently of whether the rectal temperature remains constant or decreases. Possibly this is a fatigue effect; the overworked glands are not able to maintain the osmotic difference between blood and sweat (23, 39). That there actually exists a fatigue of sweat glands could be best demonstrated by measuring the total volume of sweat under severe thermal conditions. Hourly rates of sweating decline steadily when work is done in severe heat, even in well-acclimatized individuals and even if dehydration is counteracted by unrestricted drinking of a 0.1 per cent saline solution (20). This decline is greater in humid than in dry heat (62, 63). The increase in chloride concentration with continuous sweating can be counteracted by the administration of desoxycorticosterone. Cortical extracts also reduce the sodium content but increase the potassium content (39).

The second factor influencing chloride concentration is the rate of sweating. Since the first observation by Kittsteiner (36, 37), it has been confirmed many times that the concentration of chloride increases with the rate of sweating (33). In other words, the more profuse the secretion, the greater the salt content of the sweat. The sweat-rate factor has proved to be so decisive that Locke *et al.* (50) established a chloride concentration/sweating-rate index and found that the influence of various other factors can be studied with much greater reliability if this index is measured instead of simply measuring chloride concentration (p. 266).

The third factor on which chloride concentration depends is the temperature of the skin (12, 37, 23, 66). The higher the skin surface temperature, the greater is the chloride content. Robinson *et al.* (64) have shown that not only the chloride but also the sodium concentration is significantly lower on cooled parts of the body and that

the salt concentration of the sweat can be rapidly changed locally on one hand when its skin temperature is lowered or raised. The chloride content of palmar sweat shows the same variations with changing skin temperatures (13).

The chloride content of palmar sweat differs considerably from that of eccrine sweat on other parts of the body, in so far as its concentration may considerably exceed that of the blood plasma (46). This is particularly true for the slow, intermittent type of secretion, in which sodium chloride values up to 1.64 per cent (average 1.09 per cent) were recorded. Lobitz *et al.* interpreted these conspicuously high values as being caused by reabsorption of water from the ducts, occurring when the secretion is slow and sluggish (46) (p. 190). Lobitz found that in profuse palmar sweat the mean concentration was 0.385 per cent sodium chloride. Darling (13), studying profuse sweating of palms, found in most cases less than half the chloride level of blood plasma.

The salt concentration in sweat also depends on the salt intake (36, 37). Locke *et al.* (50) have shown that at a constant rate of sweating the chloride concentration parallels the salt intake. With excess salt intake in normals, the chloride/sweating-rate index reaches unusually high values, approximating those which one finds in untreated Addisonian patients. These authors also find that the concentration of sodium in sweat rises and falls with the concentration of chlorides, and vice versa. If only the amount of sodium or only that of chloride is increased in the diet, the chloride values or the indices in sweat remain in the normal range. If a diet contains high amounts of potassium, the sodium chloride content of the sweat drops (50). One might be inclined to speculate on the possible clinical significance of considerably decreasing the sodium chloride content of the skin surface by either low sodium chloride or high potassium intake. If the salt intake is restricted, the sweat glands adjust themselves with decreased salt secretion (39). According to Conn (11), this is achieved by reabsorption

of salt. He finds that if 1.9–3.2 gm. salt are given daily and 5–9 liters of sweat are secreted, the limit for reabsorption of salt by the sweat glands is 0.25–0.35 gm. NaCl per liter (p. 190).

One of the most significant changes in chloride concentration is its rapid and substantial decrease during acclimatization to heat. This phenomenon is discussed in chapter 10, page 265.

The salt content of sweat is also influenced by the prevailing environmental temperature. In winter, sweat contains almost twice as much salt as in summer. The mean Cl^- values were found to be 63.6 mEq/l (0.226 per cent) Cl^- , or, calculated for NaCl, 0.372 per cent in winter; and 32.9 mEq/l (0.117 per cent) Cl^- , or, calculated for NaCl, 0.192 per cent in summer (14). The seasonal difference is independent of the rate of sweating; not only the absolute concentration of chloride but also the sweat chloride/sweating-rate index of Locke *et al.* (50) are much higher in winter than in summer. This peculiar "conditioning" of sweat glands in summer is possibly a special case of "acclimatization." Indeed, on repeated sweating bouts in winter the chloride concentration has a great tendency to fall, so that in 3–8 days the range of sweat chloride/sweating-rate index reaches that of summer indices. With the same number of repeated sweating bouts in summer there is no such tendency (50).

An ample supply of drinking water depresses the concentration of sweat chlorides (33). However, this is a highly insufficient and inappropriate method to spare the organism from excessive losses of chloride. If water alone is given in profuse sweating, the rate of sweating will be increased, and this will invariably involve greater salt losses. Similarly, the administration of salt without water is a dangerous procedure. Ladell (41) gives the following account of the necessity for administering salt and water simultaneously. Sweat derives from extracellular fluid, and its secretion disturbs the osmotic equilibrium. If salt is not replaced, water flows from the extracellular to the intracellular

compartments, until the osmotic pressure there decreases and the fluid content increases. When salt is given but no water at all or only slowly, the water loss is borne by the intracellular compartment, and the osmotic pressure rises in both compartments. Life becomes endangered in water deficiency by intracellular desiccation (41) and in salt deficiency by a fall in colloid osmotic pressure in the cell fluids (51).

C. DERMAL LOSS OF POTASSIUM

According to Hancock, Whitehouse, and Haldane (23), under resting conditions in a cool environment the loss of potassium through the skin is in excess of that of sodium. They found that in the wash water of an adult man 67–84 per cent of the total chlorides accounted for potassium chloride, when all the potassium was calculated as chloride. It is not known how much of this potassium derives from scales and sebum and how much from insensible sweat. With visible sweat setting in and with increasing chloride losses, the proportion of potassium to sodium gradually declines to 1:9. In other words, sweat which is not contaminated by salts derived from epidermis and sebaceous glands contains about nine times as much sodium chloride as potassium chloride (23).

Dill *et al.* (15), studying sweat electrolytes in acclimatized individuals, found that not only concentration of Na^+ and Cl^- but—to a lesser degree—that of K^+ also increases with the rate of sweating, while the nitrogen content decreases (Table 3). These authors point out that the concentration of potassium in sweat is about the same as in interstitial fluids, whereas the concentration of Na^+ and Cl^- may be as low as one-tenth the concentration in the interstitial fluids.

In diseases of the adrenal glands and in “stress” situations the sodium to potassium relationship is modified in the sweat, reflecting the electrolyte dysbalance in the blood. In adrenal insufficiency this ratio of sodium to potassium also reflects the inability to acclimatize (p. 266).

In infants the relative amounts of potas-

sium losses through the skin are greater than in adults, particularly in cases of visible sweating. Pratt *et al.* (60), analyzing skin washings of sweating infants, obtained the following ratios: 5 mM Cl^- , 6 mM K^+ , and 4 mM Na^+ .

Whereas older data indicated that potassium concentration in sweat greatly exceeds that in the blood serum (66) and that potassium losses may contribute to the symptoms of heat exhaustion (6), this is not borne out in later analyses. Still the fact remains that the potassium concentration of sweat is al-

TABLE 3 (15)

AVERAGE CONCENTRATION OF SWEAT CONSTITUENTS IN ACCLIMATIZED PERSONS AT DIFFERENT SWEATING RATES

	Rate (Cc/ Min)	N (Mg/ L)	Cl (MEq/ L)	Na (MEq/ L)	K (MEq/ L)
Low rate.....	13.1	332	25.9	23.3	2.7
High rate....	23.1	280	31.9	31.9	3.1

most as high as that of blood, while its sodium concentration is far below the blood level.

D. DERMAL LOSS OF OTHER MINERALS

1. Calcium

The calcium content of sweat is negligible. All investigators agree that at any time its concentration in sweat is much lower than in blood serum and that as sweating progresses, its concentration decreases further. From early data it appears that the concentration can be as low as 0.3 mg. per cent and hardly ever exceeds 5 mg. per cent (93). Mitchell and Hamilton (55) found an initial value of 2.94 mg. per cent, but later in the course of sweating only 0.88 mg. per cent. Still the daily dermal loss of calcium under conditions of minimal sweating seems to be comparable to that of chlorides: it was estimated to be 149 mg. (55). This would indicate that although loss with sweat is minimal, loss with epidermal debris is relatively quite high.

2. Magnesium

Small amounts of magnesium are also present in eccrine sweat. Apparently, there is no constant relationship between the calcium and magnesium content (93, 27). The average quotient Ca/Mg is 5:1. The range of magnesium concentration found by Talbert *et al.* (93) was 0.14–4.5 mg/100 cc. Both calcium and magnesium may be entirely absent in sweat (60). Pratt *et al.* stated that in infants cutaneous loss of calcium and phosphorus is negligible (60).

3. Sulfates and Phosphates

Older data reveal the presence of sulfate and phosphate ions in eccrine sweat (66). The newer literature on the amount of sulfur and phosphorus in sweat was reviewed by Spector, Hamilton, and Mitchell (86). Talbert (94) found a phosphorus range varying between traces and 4.8 mg/100 cc; but Mitchell's maximal value had been only 0.22 mg/100 cc (55). Talbert (94) found more phosphorus in work sweat than in heat sweat and an increased amount of phosphorus after administration of a diet high in phosphorus.

4. Iron

Mitchell and Hamilton (55, 16) calculated the daily average dermal loss of iron to be 6.5 mg. under conditions of minimal sweating and an increase up to ten times under conditions of profuse sweating. In visible sweat its concentration was 1–2 mg/l, independently of the rate of sweating. Adams *et al.* (1) found 6–10 mg Fe/l in unfiltered opalescent sweat; but from the supernatant of centrifuged samples they recovered only 5 per cent of the original amount. The conclusion was arrived at that iron is contained in particulate matter and, therefore, that dermal loss of iron is not a

sweat-gland function but is due to epidermal desquamation.

Of course, it should be considered that apocrine glands may well participate in the excretion of iron-containing corpuscular elements. On the other hand, the relatively high iron content of cornified epithelial cells has been known for a long time (66), and there can be little doubt that dermal loss of iron is chiefly due to epithelial disintegration. Dermal loss of iron does not depend on the iron intake (55).

The relatively large dermal loss of iron, no matter whether by glandular activity or by desquamation, is unexpected in view of the well-established fact that the body does not absorb significant amounts of iron, except when the iron stores are depleted (22), and that only insignificant amounts of iron escape into the urine and bile (21). Iron deriving from red cell decomposition is reutilized by the organism (35). In other words, the adult organism has a stable stock of iron; intake and output are at an extreme minimum. With these facts in mind, Whipple *et al.* (87) re-examined the problem of iron excretion through the skin by administering, orally and intravenously, radioiron to dogs and analyzing skin washings obtained by thorough scrubbing in a baby bathinette and by using tap water and a commercial detergent. In this way little or no radioiron could be removed from skin and hair, an indication that no significant amounts of iron are excreted through the skin. This is in contradistinction to the results of Mitchell and Hamilton (55) and of Adams *et al.* (1).²

² If radioiron is injected into rats, there is considerable storage in the skin (personal communication of Drs. J. Eldredge and W. F. Bethard; to be published).

BIBLIOGRAPHY

1. ADAMS, W. S.; LESLIE, A.; and LEVIN, M. H. The dermal loss of iron, *Proc. Soc. Exper. Biol. & Med.*, **74**:46–48, 1950.
2. ADOLPH, E. F. *Physiology of man in the desert*. New York: Interscience Publishers, Inc., 1947.
3. ARN, K. D., and REIMER, A. Minimal sodium losses through the skin, *J. Clin. Investigation*, **29**:1342–46, 1950.
4. BERGEIM, O., and CORNBLEET, T. *Acidity*

- of the scalp; nature and possible relation to seborrhea, *Arch. Dermat. & Syph.*, **56**: 448-51, 1947.
5. BOLLIGER, A. Water extractable constituents of hair, *J. Invest. Dermat.*, **17**:79-84, 1951.
 6. BORCHARDT, W. Zur Physiologie und physiologischen Chemie des Schwitzens, zugleich ein Beitrag zur allgemeinen Physiologie der Drüsentätigkeit, *Arch. f. d. ges. Physiol.*, **214**:169-88, 1926.
 7. BOST, R. W., and BORGSTROM, P. Cutaneous excretion of nitrogenous material in New Orleans, *Am. J. Physiol.*, **79**:242-44, 1926.
 8. BURCKHARDT, W., and BÄUMLE, W. Die Beziehungen der Säureempfindlichkeit zur Alkaliempfindlichkeit der Haut, *Dermatologica*, **102**:294-300, 1951.
 9. CARRIÉ, C., and HEEMEYER, R. Untersuchungen über die chemischen Substanzen auf der Haut. II. Über das Vorkommen von Kochsalz und Cholesterin auf der Haut, *Arch. f. Dermat. u. Syph.*, **173**: 606-11, 1936.
 10. CARRIÉ, C., and KOENIG, R. Untersuchungen über die chemischen Substanzen auf der Haut. III. Über den Zuckergehalt auf der Haut bei Normalen und Diabetikern, *ibid.*, pp. 611-14.
 11. CONN, J. W. The mechanism of acclimatization to heat. *In*: *Advances in internal medicine*, **3**:373-93. New York: Interscience Publishers, Inc., 1949.
 12. CRAMER, E. Über die Beziehung der Kleidung zur Hauttätigkeit, *Arch. f. Hyg.*, **10**: 231-82, 1890.
 13. DARLING, R. C. Some factors regulating the composition and formation of human sweat, *Arch. Phys. Med.*, **29**:150-55, 1948.
 14. DAVIES, D. F., and CLARK, H. E. Electrolyte concentration of sweat as a differential diagnostic test in hypertension, *Am. J. Physiol.*, **159**:566-67, 1949 (abstr.).
 15. DILL, D. B.; HALL, F. G.; and EDWARDS, H. T. Changes in composition of sweat during acclimatization to heat, *Am. J. Physiol.*, **123**:412-19, 1938.
 16. Editorial. Dermal loss of iron, *J.A.M.A.*, **144**:47, 1950.
 17. EMBDEN, G. M., and TACHAU, H. Über das Vorkommen von Serin im menschlichen Schweiß, *Biochem. Ztschr.*, **28**:230-36, 1910.
 18. FISHBERG, E. H., and BIERMAN, W. Acid-base balance in sweat, *J. Biol. Chem.*, **97**: 433-41, 1932.
 19. GAD-ANDRESEN, K. L. Die Verteilung des Harnstoffes im Organismus, *Biochem. Ztschr.*, **116**:266-302, 1921.
 20. GERKING, S. D., and ROBINSON, S. Decline in the rates of sweating of man working in extreme heat, *Am. J. Physiol.*, **147**:370-78, 1946.
 21. HAHN, P. F.; BALE, W. F.; HETTIG, R. A.; KAMEN, M. D.; and WHIPPLE, G. H. Radioactive iron and its excretion in urine, bile, and feces, *J. Exper. Med.*, **70**:443-51, 1939.
 22. HAHN, P. F.; BALE, W. F.; LAWRENCE, E. O.; and WHIPPLE, G. H. Radioactive iron and its metabolism in anemia; its absorption, transportation and utilization, *J. Exper. Med.*, **69**:739-53, 1939.
 23. HANCOCK, W.; WHITEHOUSE, A. G. R.; and HALDANE, J. S. The loss of water and salts through the skin, and corresponding physiologic adjustments, *Proc. Roy. Soc. London, s.B.*, **105**:43-59, 1929.
 24. HAUGEN, C. O., and TALBERT, G. A. Simultaneous study of the constituents of the sweat, urine and blood, also gastric acidity and other manifestations resulting from sweating. VII. Amino acids, *Am. J. Physiol.*, **85**:224-28, 1928.
 25. HAWK, P. B.; OSEER, B. L.; and SUMMERSON, W. H. *Practical physiological chemistry*. 12th ed. Philadelphia: Blakiston Co., 1947.
 26. HIER, S. W.; CORNBLEET, T.; and BERGEIM, O. The amino-acids of human sweat, *J. Biol. Chem.*, **166**:327-33, 1946.
 27. HOPF, G. Chemisch-physikalische Untersuchungen über den menschlichen Schweiß, *Arch. f. Dermat. u. Syph.*, **171**: 301-12, 1935.
 28. JOHNSON, B. C.; HAMILTON, T. S.; and MITCHELL, H. H. Excretion of pyridoxine, "pseudopyridoxine" and 4-pyridoxic acid in urine and sweat of normal individuals, *J. Biol. Chem.*, **158**:619-23, 1945.
 29. ———. Effect of choline intake and environmental temperature on the excretion of choline from the human body, *ibid.*, **159**:5-8, 1945.
 30. ———. Excretion of nicotinic acid, nicotinamide, nicotinuric acid and *N'* methyl-nicotinamide by normal individuals, *ibid.*, pp. 231-36.
 31. ———. Excretion of "folic acid" through

- the skin and in the urine of normal individuals, *ibid.*, pp. 425-29.
32. JOHNSON, B. C.; MITCHELL, H. H.; and HAMILTON, T. S. Occurrence of inositol and of *p*-aminobenzoic acid in sweat, *J. Biol. Chem.*, **161**:357-60, 1945.
 33. JOHNSON, R. E.; PITTS, G. C.; and CONSOLAZIO, F. C. Factors influencing chloride concentration in human sweat, *Am. J. Physiol.*, **141**:575-89, 1944.
 34. KARITZKY, B. Vegetative Belastung und Schweissfunktion, *Ztschr. f. d. ges. inn. Med.*, **2**:555-61, 1947.
 35. KINNEY, T. D.; HEGSTED, D. M.; and FINCH, C. A. Influence of diet on iron absorption. I. The pathology of iron excess, *J. Exper. Med.*, **90**:137-46, 1949.
 36. KITTSTEINER, C. Sekretion, Kochsalzgehalt und Reaktion des Schweißes, *Arch. f. Hyg.*, **73**:275-306, 1911.
 37. ———. Weitere Beiträge zur Physiologie der Schweißdrüsen und des Schweißes, *ibid.*, **78**:275-326, 1913.
 38. ———. Physiologische Chemie des Schweißes, *Dermat. Wchnschr.*, **62**:553-58, 1916.
 39. LADELL, W. S. S. Thermal sweating, *Brit. M. Bull.*, **3**:175-79, 1945.
 40. ———. Creatinine losses in the sweat during work in hot humid environments, *J. Physiol.*, **106**:237-44, 1947.
 41. ———. The changes in water and chloride distribution during heavy sweating, *ibid.*, **108**:440-50, 1949.
 42. LOBITZ, W. C., and MASON, H. L. Chemistry of palmar sweat. V. Ammonia nitrogen, *Arch. Dermat. & Syph.*, **57**:69-73, 1948.
 43. ———. Chemistry of palmar sweat. VI. Uric acid, *ibid.*, pp. 387-91.
 44. ———. Chemistry of palmar sweat. VII. Discussion of studies on chloride, urea, glucose, uric acid, ammonia nitrogen and creatinine, *ibid.*, pp. 907-15.
 45. LOBITZ, W. C., and OSTERBERG, A. E. Chemistry of palmar sweat. I. Preliminary report. Apparatus and techniques, *J. Invest. Dermat.*, **6**:63-74, 1945.
 46. ———. Chemistry of palmar sweat. II. Chloride, *Arch. Dermat. & Syph.*, **56**:462-67, 1947.
 47. ———. Chemistry of palmar sweat. III. Reducing substances (glucose), *ibid.*, pp. 819-26.
 48. ———. Chemistry of palmar sweat. IV. Urea, *ibid.*, pp. 827-33.
 49. ———. Use of the spectrophotometer in capillary tube colorimetry for determination of reducing substances (glucose, chlorides, ammonia nitrogen, uric acid, and creatinine), *J. Invest. Dermat.*, **7**:135-44, 1946.
 50. LOCKE, W.; TALBOT, N. B.; JONES, H. S.; and WORCESTER, J.: Studies on the combined use of measurements of sweat electrolyte composition and rate of sweating as an index of adrenal cortical activity, *J. Clin. Investigation*, **30**:325-37, 1951.
 51. McCANCE, H. A. Experimental sodium chloride deficiency in man, *Proc. Roy. Soc. London, s.B.*, **119**:245-68, 1935.
 52. McSWINEY, B. A. The composition of human perspiration, *Proc. Roy. Soc. Med.*, **27**:839-48, 1934.
 53. MARCHIONINI, A., and OTTENSTEIN, B. Stoffwechseleränderungen im Schwitzbad bei Hautgesunden und Hautkranken, *Klin. Wchnschr.*, **10**:969-74, 1931.
 54. MICKELSEN, O., and KEYS, A. The composition of sweat with special reference to the vitamins, *J. Biol. Chem.*, **149**:479-90, 1943.
 55. MITCHELL, H. H., and HAMILTON, T. S. The dermal excretion under controlled environmental conditions of nitrogen and minerals in human subjects with particular reference to calcium and iron, *J. Biol. Chem.*, **178**:345-61, 1949.
 56. MOORE, D. D.; LAVIETES, P. H.; WAKEMAN, A. M.; and PETERS, J. P. The effect of ingested urea on nitrogen metabolism, *J. Biol. Chem.*, **91**:373-85, 1931.
 57. MOSHER, H. H. Simultaneous study of constituents of urine and perspiration, *J. Biol. Chem.*, **99**:781-89, 1937.
 58. OTTENSTEIN, B. Beitrag zur Chemie des Schweißes, *Arch. f. Dermat. u. Syph.*, **191**:116-22, 1950.
 59. PETERS, J. P. Body water: the exchange of fluids in man. Springfield, Ill.: Charles C Thomas, 1935.
 60. PRATT, E. L.; COOKE, R. E.; and DARROW, D. C. Water and electrolyte losses from skin of infants, *Federation Proc.*, **8**:237-38, 1949.
 61. REYNOLDS, T. Sweat sodium levels in congestive heart failure, *Proc. Soc. Exper. Biol. & Med.*, **79**:118-21, 1952.
 62. ROBINSON, S. The control of sweating in working men, *Federation Proc.*, **6**:190-91, 1947.

63. ROBINSON, S., and GERKING, S. D. Thermal balance of men working in severe heat, *Am. J. Physiol.*, **149**:476-88, 1947.
64. ROBINSON, S.; GERKING, S. D.; TARRELL, E. S.; and KINCAID, R. K. Effect of skin temperature on salt concentration of sweat, *J. Appl. Physiol.*, **2**:654-62, 1950.
65. ROTHMAN, S. Hautabscheidungen. In: OPPENHEIMER, Handb. d. Biochem. d. Menschen u. d. Tiere, suppl. vol., **2**:533-41. 2d ed. Jena: G. Fischer, 1934.
66. ROTHMAN, S., and SCHAFF, F. Chemie der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1**/2:161-377. Berlin: J. Springer, 1929.
67. ROTHMAN, S.; SMILJANIC, A. M.; and MURPHY, J. C. The nitrogenous material on normal human skin surface, *J. Invest. Dermat.*, **13**:317-18, 1949.
68. ROTHMAN, S., and SULLIVAN, M. B. Amino acids on the normal skin surface, *J. Invest. Dermat.*, **13**:319-21, 1949.
69. SAIKI, A. K.; OLMANSON, G.; and TALBERT, G. A. Simultaneous study of the constituents of the sweat, urine and blood, also gastric acidity and other manifestations resulting from sweating. IX. Uric and lactic acids, *Am. J. Physiol.*, **100**:328-30, 1932.
70. SAIKI, A. K., and TALBERT, G. A. Uric acid in the sweat of normal subjects, *Am. J. Physiol.*, **85**:404, 1928.
71. SARGENT, F.; ROBINSON, P.; and JOHNSON, R. E. Water soluble vitamins in sweat, *J. Biol. Chem.*, **153**:285-94, 1944.
72. SCHENK, P. Kraftstoffwechsel im Sport. Sportärztetagung d. Dt. Ärztebundes z. Förderg. d. Leibesübgn., 1925, Verhandlungsbericht, p. 91. Jena: G. Fischer, 1926.
73. SCHENK, P., and WISSEMAN, M. Der Marathonläufer, *Med. Klin.*, **22**:643-45 and 683-86, 1926.
74. SCHEUNERT, A.; KLEIN, W.; and STEUBER, M. Über die Verwertbarkeit des Harnstoffs als Eiweissquelle für Wiederkäuer, zugleich ein Beitrag zur Frage der exkretorischen Funktionen der Haut, *Biochem. Ztschr.*, **133**:137-91, 1922.
75. SCHLEGEL, J. U. Fluctuations of serum choline in women, *Am. J. Physiol.*, **158**:345-50, 1949.
76. SCHULZE, W. Über den Zuckergehalt auf der Haut, im Hautdialysat und im Schweiß, *Arch. f. Dermat. u. Syph.*, **181**:471-85, 1940.
77. SCHULZE, W., and KUNZ, K. Über den Anteil des als Osazon bestimmte Zuckers und einiger anderer Substanzen am Reduktionsvermögen des Schweißes, *Arch. f. Dermat. u. Syph.*, **181**:486-94, 1940.
78. SCHUMANN, R. Über das Vorkommen von Kreatinin und Kreatin im menschlichen Schweiß, *Ztschr. f. d. ges. exper. Med.*, **79**:145-52, 1931.
79. SCHWARTZ, I. L.; THAYSEN, J. H.; and DOLE, V. P. Urea excretion in human sweat as a tracer for movement of water within the secreting gland, *J. Exper. Med.*, **97**:429-37, 1953.
80. SCHWENKENBECHER, A. Die Haut als Exkretionsorgan. In: BETHE, Handb. d. norm. u. path. Physiol., **4**:709-68. Berlin: J. Springer, 1929.
81. SCHWENKENBECHER, A., and SPITTA, O. Über die Ausscheidung von Kochsalz und Stickstoff durch die Haut, *Arch. f. exper. Path. u. Pharmakol.*, **56**:284-300, 1906-7.
82. SIEBURG, E., and PATZSCHKE, W. Menstruation und Cholinstoffwechsel, *Ztschr. f. d. ges. exper. Med.*, **36**:324-43, 1923.
83. SILVERS, S.; FORSTER, W.; and TALBERT, G. A. Simultaneous study of the constituents of sweat, urine and blood, also gastric acidity and other manifestations resulting from sweating. VI. Sugar, *Am. J. Physiol.*, **84**:577-82, 1928.
84. SNAPPER, L., and GRÜNBAUM, A. Über die Milchsäureausscheidung im Harn und Schweiß während Fussballwettkämpfen, *Biochem. Ztschr.*, **206**:319-33, 1929.
85. ———. Über Milchsäureausscheidung im Harn und Schweiß bei verschiedenen Sportarten, *ibid.*, **208**:212-20, 1929.
86. SPECTOR, H.; HAMILTON, T. S.; and MITCHELL, H. H. The effect of pantothenic acid dosage and environmental temperature and humidity upon dermal and renal excretion of pantothenic acid, *J. Biol. Chem.*, **161**:145-52, 1945.
87. STEWART, W. B.; SNOWMAN, R. T.; YUILE, C. L.; and WHIPPLE, G. H. Radioiron excretion by skin and kidney of dogs, *Proc. Soc. Exper. Biol. & Med.*, **73**:473-75, 1950.
88. TALBERT, G. A., and HAUGEN, C. O. Simultaneous studies of the constituents of sweat, urine and blood, also gastric acidity and other manifestations resulting from sweating. I. Chlorides, *Am. J. Physiol.*, **81**:74-80, 1927.
89. TALBERT, G. A.; SILVERS, S.; and JOHN-

- SON, W. Simultaneous studies of the constituents of sweat, urine and blood. . . . II. Total nitrogen of sweat and urine; total non-protein nitrogen of blood, *Am. J. Physiol.*, **81**:81-85, 1927.
90. TALBERT, G. A.; FINKLE, J. R.; and KATSUKI, S. S. Simultaneous studies of the constituents of sweat, urine and blood. . . . III. Urea, *Am. J. Physiol.*, **82**:153-56, 1927.
 91. TALBERT, G. A.; FINKLE, R.; and KATSUKI, D. Simultaneous studies of the constituents of sweat, urine and blood. . . . IV. Ammonia nitrogen, *Am. J. Physiol.*, **82**: 639-43, 1927.
 92. TALBERT, G. A., and ROSENBERG, I. Simultaneous studies of the constituents of sweat, urine and blood. . . . V. Gastric acidity, *Am. J. Physiol.*, **84**:520-23, 1928.
 93. TALBERT, G. A.; HAUGEN, C.; CARPENTER, R.; and BRYANT, J. E. Simultaneous studies of the constituents of sweat, urine and blood. . . . X. Basic metals, *Am. J. Physiol.*, **104**:441-42, 1933.
 94. TALBERT, G. A.; STINCHFIELD, F.; and STAFF, H. Simultaneous studies of the constituents of sweat, urine and blood. . . . XI. Phosphorus and sulphur, *Am. J. Physiol.*, **106**:488-90, 1933.
 95. TALBERT, G. A.; TYVAND, R.; GOEHL, R.; and GRONHOVD, G. Changes noted in the specific gravity, hemoglobin and corpuscle count of the blood after profuse sweating, *Am. J. Physiol.*, **85**:409, 1928.
 96. THOMAS, K. Über die biologische Wertigkeit der Stickstoffsubstanzen in verschiedenen Nahrungsmitteln; Beiträge zur Frage nach dem physiologischen Stickstoffminimum, *Arch. f. Physiol.*, pp. 219-302, 1909.
 97. THURMON, F. M., and OTTENSTEIN, B. Studies on the chemistry of human perspiration with special reference to its lactic acid content, *J. Invest. Dermat.*, **18**: 333-39, 1952.
 98. USHER, B., and RABINOWITCH, I. M. Excretion of sugars in sweat; its relationship to eczema, *Arch. Dermat. & Syph.*, **16**: 706-13, 1927.
 99. VAN SCOTT, E. J. Personal communication.
 100. VERMEER, D. J. H.; JONG, J. C. DE; and LENSTRA, J. B. The significance of amino-acids for the neutralization by the skin, *Dermatologica*, **103**:1-18, 1951.
 101. VOIT, E. Über die Grosse der Erneuerung der Horngebilde beim Menschen. I. Die Haare, *Ztschr. f. Biol.*, **90**:508-24, 1930.
 102. ———. Über die Grosse der Erneuerung der Horngebilde beim Menschen. II. Die Nägel, *ibid.*, pp. 525-48.
 103. ———. Über die Grosse der Erneuerung der Horngebilde beim Menschen. III. Die Oberhaut, *ibid.*, pp. 549-56.
 104. WEINER, J. S., and VAN HEYNINGEN, R. Lactic acid and sweat gland function, *Nature*, **164**:351-52, 1949.
 105. ———. Comparison of arm and general body sweat, *J. Physiol.*, **112**:13P, 1951.
 106. WHITEHOUSE, A. G. R. The dissolved constituents of human sweat, *Proc. Roy. Soc. London, s.B.*, **117**:139-54, 1935.
 107. WILLIAMS, J. W. The pathological physiology of the kidneys (and sweat glands) in infections, *Urol. & Cutan. Rev.*, **44**:47-49, 1940.

CHAPTER 8

pH of Sweat and Skin Surface

I. MARCHIONINI'S "ACID MANTLE"	221
II. pH OF SWEAT	222
III. pH OF THE HEALTHY SKIN SURFACE	222
IV. pH OF THE SURFACE IN DISEASES	224
V. BUFFERING CAPACITY	227

I. MARCHIONINI'S "ACID MANTLE"

THE discovery of the acid nature of freshly secreted eccrine sweat and of the intact human skin surface, the first application of electrometric methods of estimating hydrogen-ion concentration on the skin surface, the development of the concept of the "acid mantle," and discussion of its possible significance in the pathogenesis of dermatoses have often been reviewed (50, 31, 19, 1).

In the aqueous film on most parts of the human skin surface, Schade and Marchionini (51, 52) found a pH range of 3.0–5.0. This acid reaction was interpreted as being due to impregnation of the horny layer with acid constituents of eccrine sweat. It was stated that, although the pH of the skin surface first becomes less acid with the onset of sweating (pH 5–6), this is because the weakly acid sweat dilutes the higher acid concentration on the surface. But with progressive evaporation of sweat-water, a highly acid reaction again results. The increasing acidity with evaporation of water from sweat was observed also *in vitro* (38). In intertriginous areas, where sweat evaporation is hindered, and in regions supplied by apocrine glands the reaction was found to be less acid or neutral (35, 36). The pH of ther-

mal eccrine sweat was found to be 3.8–5.6; that of sweat from areas supplied by apocrine glands, 6.2–6.9 (38). When apocrine sweat was evaporated, it became more alkaline instead of more acid, as did eccrine sweat.

In addition, Marchionini (35) found that the predilectional areas of seborrheic dermatitis, such as the mid-lines on the chest and back, are less acid than other nonintertriginous areas. He placed great emphasis on the possible role of acid pH in the natural ability of the skin to resist invasion by bacteria and fungi (51, 52) and assumed that intertriginous areas are particularly vulnerable to such infections because of their relatively high surface pH's. He dealt with prevention and therapy of interdigital fungus infections by local application of acids and thought he obtained good results (39; see also 55).

Whereas the conclusions of Marchionini on the significance of the surface pH in the pathogenesis and localization of skin diseases is still controversial, his experimental data have been essentially confirmed many times (20, 19, 33, 34), even if the average pH of the surface proved to be less acid than stated originally.

II. pH OF SWEAT

Freshly secreted eccrine thermal sweat is always acid in its reaction, even if sometimes less acid than Marchionini's original data indicated. Talbert *et al.* (64) and Levin-Silvers (31) found an average pH of 5.73; Hardt and Palmer (17), an average of 6.49. As sweating progresses, the acidity mostly decreases (28, 27). This is true also when sweating is produced intermittently in subsequent periods (17). However, Dill *et al.* (13) also observed the opposite behavior in some cases: the sweat became more acid in subsequent bouts. Some data indicated reciprocal behavior of chloride content and acidity: the higher the salt content, the less the acidity (28).

In any case, quite considerable amounts of acid may be lost with profuse thermal sweating. It is controversial, however, whether this has any repercussion on the acid-base balance of the body as a whole, namely, whether it has any base-saving value and/or whether it may lead to alkalosis (16, 17, 13) (p. 211).

The question of which compounds are responsible for the acidity of eccrine sweat is undecided. Three compounds or groups of compounds must be considered: (1) Lactic acid was claimed to be responsible by many (16, 49, 3, 66), because the lactic acid content and the acidity parallel each other (44) and because of the good buffering capacity of the lactic acid/lactate system at pH 4–5 (16). (2) Dicarboxylic amino acids could participate. Both glutamic and aspartic acids occur in sweat, and the sum of their molar concentrations seems to be greater than that of the basic amino acids (23). (3) The normal fatty acids, which lie on the skin surface (pp. 317 ff.) and which may be

taken up by the outpouring sweat, can influence the pH of sweat so far as they are water-soluble. The great bulk of the free fatty acids on the skin surface, however, are high-molecular acids, whose water-solubility is negligible. Propionic, butyric, pentanoic, and hexanoic acids, as far as they are present, may contribute. It is conspicuous, however, that removal of fatty acids by fat solvents does not essentially influence the pH of the skin surface (4, 5, 6, 7, 32). Therefore, they could hardly influence that of sweat.

If eccrine sweat is elicited by means other than external heat, the acidity is less pronounced. Talbert (64) found an average pH of 6.4 in sweat after exercise, as contrasted with pH 5.7 in thermal sweat. According to Marchionini (38), the pH of sweat elicited by systemic administration of pilocarpine varies and is sometimes alkaline; apocrine sweat elicited by pilocarpine injections is always on the alkaline side.

Hopf (24) succeeded in alkalinizing sweat by internal administration of large doses of sodium bicarbonate and acidifying it by giving ammonium chloride or acid sodium phosphate. The pH of eccrine sweat was easier to influence by this method than was apocrine sweat, the original pH of which was much less acid or was even alkaline. But even eccrine sweat did not react so promptly to these alkalinizing and acidifying agents as did the urine. Shelley and Hurley (60), who probed single apocrine sweat ducts, found the pH of apocrine sweat to range between 5.0 and 6.5, while that of eccrine sweat was between 4 and 6. The apocrine sweat was more alkaline by only pH 0.5; and even this difference was not quite certain.

III. pH OF THE HEALTHY SKIN SURFACE

Precise and reliable potentiometric measurements on the arms of normal males, females, and children were carried out by I. H. Blank (4, 5, 6, 7) with the glass electrode. He confirmed that the pH of the

aqueous film on the skin surface is always on the acid side, varying from 4 to 7 but mostly being between 4.2 and 5.6. He also reported the following significant findings: females average 0.5 unit higher than males; in adult

males the pH is lower than in prepubertal boys, while female adults have averages almost identical with those of prepubertal girls; there are greater individual spread and greater diurnal variation in the female than in the male, which are apparently independent of the menstrual cycle and of atmospheric temperature.

Draize (14), working with a technic similar to Blank's, arrived at similar results, except that he found, as Marchionini (37) had before him, some suggestion of cyclic changes in women from the onset of menstruation through the third week of the cycle there was a slight rise, followed by a drop during the fourth week of the cycle to a level below that of the first week of the cycle. He also examined Negro males and found their skin surface pH higher than that of white males, but lower than that of white females. Furthermore, he examined the pH of the skin surface in a number of laboratory animals and found it throughout to be more alkaline than in man, the values in the guinea pig coming relatively closest to the range in man. This higher alkalinity in animals was not due to dirt: washing with water or with water and soap, and thorough rinsing did not alter the values. Draize's results are reproduced in Table 1 and in Figure 1. The alkalinity of sweat in mammals (pH 7.8–8.9) other than man was noted long ago by F. N. Schultz (56) and by Korkisch (30). The more alkaline values in animals support the view that the strongly acid surface in man is at least in part created by eccrine-gland activity, which is lacking in animals. Their sweat corresponds with the apocrine sweat of man.

The measurements of P. W. Schmidt (54) with the quinhydrone electrode are comparable to those of Blank (4). He obtained on the dorsa of forearms of adult men and women an over-all average value of pH 5.46, with a spread of 4.5–6.0 in men and 5.0–6.0 in women.

All recent data confirm the acidity of the surface, but most ranges and averages show less acidity than that shown in the original communication of Marchionini. Most values

are around pH 5, and no values below pH 4 have been reported (12, 46). Marchionini himself, working with the quinhydrone electrode in 1938, reported on values not lower than pH 5 (42).

Herrmann *et al.* (19), working with an indicator method, paid particular attention to the pH in the axilla and on the scalp. They found the average pH on the scalp to be the same as on the chest and on the extensor aspect of the forearm, the average figures in all age groups being near 4. In the

TABLE 1
NORMAL pH RANGES OF SKIN OF MAN AND
COMMON LABORATORY ANIMALS (14)

Subject*	Extreme Limits of Variation	Range for Approximately 95 Per Cent of Readings	General Averages for the Various Subjects
White male.	4.04–6.26	4.2–5.8	4.85
White female.	4.70–6.66	4.8–6.2	5.50
Negro male.	4.39–6.63	4.4–6.0	5.21
Guinea pig.	4.26–7.17	4.8–6.6	5.50
Monkey.	5.50–7.52	5.8–7.0	6.42
Cat.	5.57–7.44	5.8–7.2	6.43
Rat.	5.74–7.51	5.8–7.2	6.48
Rabbit.	5.97–7.50	6.0–7.4	6.71
Dog.	5.18–9.18	6.2–8.6	7.52

* Determinations were made on 51 white males, 52 white females, and 25 Negro males. Areas chosen for examination in man were the back of the hand, inner aspect of the forearm, antecubital fossa, upper arm, cheek, and forehead; and in the animals the upper abdomen, lower abdomen, axilla, inguinal region, back, and nape of the neck.

axilla an almost linear rise in pH was noted with age from infancy on, with an additional increment in puberty. Further alkalization continued long after the conventionally assumed end of puberty. One may assume that, like the development of sebaceous glands, that of apocrine glands also continues for some time after the age of "adolescence."

Regionally there seems to be little difference in the surface pH in areas supplied by eccrine sweat glands only. But lower acidity in intertriginous areas and in other areas where sweat does not evaporate (also on soles of feet [40]) has remained an often confirmed fact. Herrmann *et al.* (19) have

confirmed the alkalinity of interdigital spaces of the feet.

In addition to eccrine sweat constituents (lactic acid, normal fatty acids, dicarboxylic amino acids), the "acidity" of keratin in the horny layer was also considered to con-

tribute to the surface acidity (59). The poor water-solubility of keratin, however, makes it improbable that it has any measurable influence. Its possible role in the buffering capacity of the surface will be discussed later.

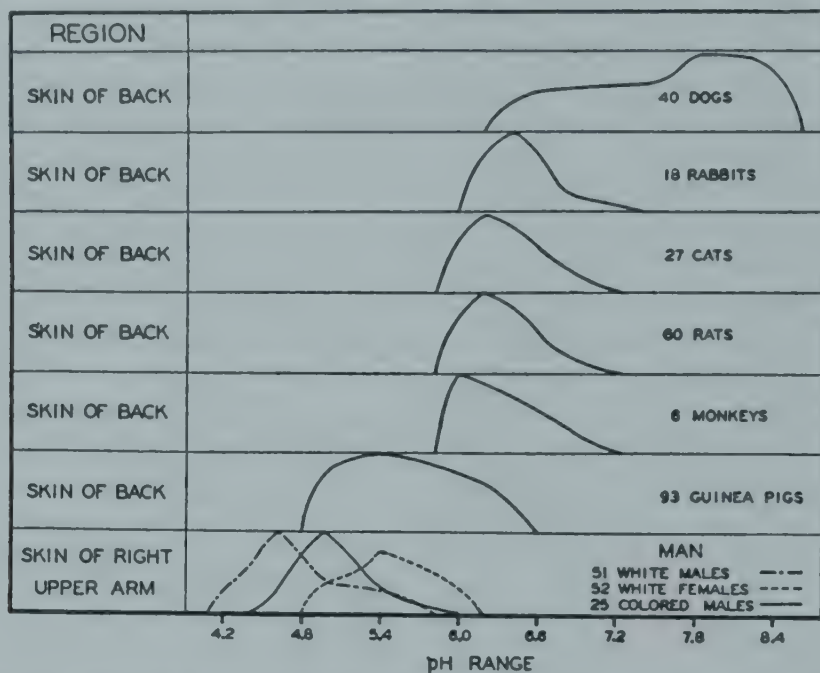


FIG. 1.—Normal pH ranges of skin of man and common laboratory animals. From Draize (14). (Reproduced by permission of the Williams & Wilkins Company.)

IV. pH OF THE SURFACE IN DISEASES

There are two different pathological aspects of the surface acidity. One is that alterations in the surface pH regionally or constitutionally may predispose to disease, and the other is that the disease process in the skin may alter the surface pH. Marchionini (35) stressed the first possibility, but the results of his prophylactic and therapeutic attempts to keep these surfaces acid were rather modest, and direct evidence for the role of low acidity in bacterial and fungus infections was not presented. The whole situation in intertriginous areas is different also in so many other respects (e.g., higher skin temperature, maceration of horny layer, more intense mechanical stress, etc.) that it is difficult to evaluate the role of single factors. Schmidt (54) pointed out that the pH of Sabouraud's medium, in

which pathogenic hyphomycetes grow so well, is 4.9, hardly less acid than the average skin surface.

Marchionini (35) emphasized the low acidity of all—intertriginous and nonintertriginous—predilectional sites of seborrheic dermatitis. Recently, with glass-electrode measurements, Anderson (1) found that in children with seborrheic dermatitis the pH is raised all over the body surface (Fig. 2 and Table 2). Thus one has to consider seriously the question as to whether or not this high pH is part of the constitutional anomaly which predisposes to seborrheic dermatitis (kerosis of Darier). These patients' ability to sweat has never been compared with that of normal persons. It is, however, an empirical fact that the uninvolved skin of these patients is "dry" rather than greasy, as the

name would indicate, and that they do not well tolerate excessive use of water and soap. All this seems to make it worth while to study in greater detail the sweating ability, sebaceous excretions, and surface pH of un-

which is relieved by washing. The pH on the surface of of inflammatory lesions seems not to be altered substantially as long as the superficial barrier which hinders diffusion of alkaline (or neutral) extracellular fluid to

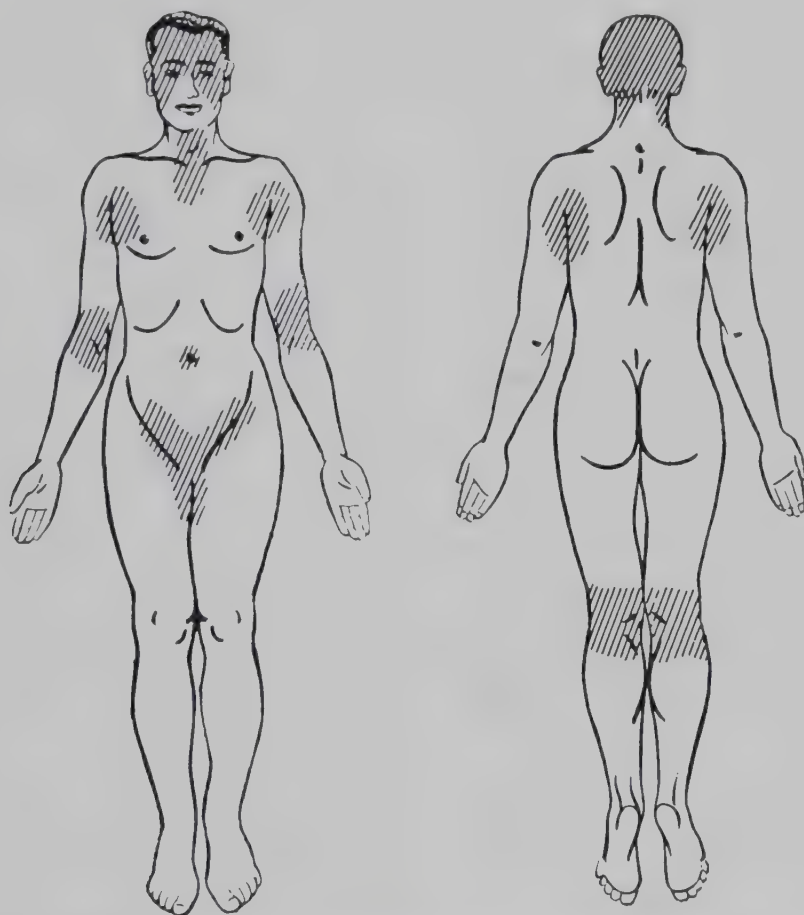


FIG. 2.—Areas of highest pH on the bodies of children with seborrheic eczema. Glass-electrode measurements. From Anderson (1). (Reproduced by permission of the author and H. K. Lewis & Company, Ltd.)

involved skin in these patients than has been done hitherto.

Even more significant is a finding of Anderson (1) on "dry" or xerodermatic skin, a type which is often associated with Besnier's prurigo (atopic dermatitis): on dry skin the pH tends to be raised over the whole body surface (Fig. 3 and Table 2). This might be explained by the newer concept of "synergism" (21) of sweat and sebaceous glands. Possibly in "dry" skin not only sebaceous excretion but also sweat secretion is reduced (p. 294).

High acidity of the scalp was thought to be sometimes associated with itching (3),

TABLE 2
AVERAGE pH VALUES OF THE SKIN SURFACE
UNDER A VARIETY OF CONDITIONS (1)

	Normal	Seborrheic Eczema	Besnier's Prurigo
Dorsum of hand	5.04	5.71	5.35
Palm of hand	5.25	5.77	5.52
Flexor surface of wrist	5.33	5.74	5.63
Antecubital fossa	5.44	6.24	5.74
Axilla	5.45	6.33	5.84
Cheek	5.28	6.42	5.95
Scalp	5.00	6.45	5.52
Retroauricular fold	4.87	6.28	5.34
Sole of foot	5.25	5.47	5.33
Popliteal fossa	5.53	6.48	5.87

the surface is not broken. Scholtz (55) found acid values on top of inflammatory papules and scaling lesions not exceeding pH 6.1. In chronic lichenified and scaling "eczemas," normal values or very little alteration was noted by Lustig and Perutz (34) and by Levin and Silvers (31).

However, as soon as the barrier is injured, as is the case in acute eczematous derma-

around pH 7 or higher. However, Marchionini (40) found that this is not true for vesicles in true (nonmycotic) dysidrosis, in which the pH of vesicles averages 5, as contrasted with vesicles caused by staphylococci or fungi (pH 7) or with vesicles of -id reactions (pH 7.2-8.2). As the turning point of the color change of litmus is at pH 6.8, Marchionini (40) felt that testing of the

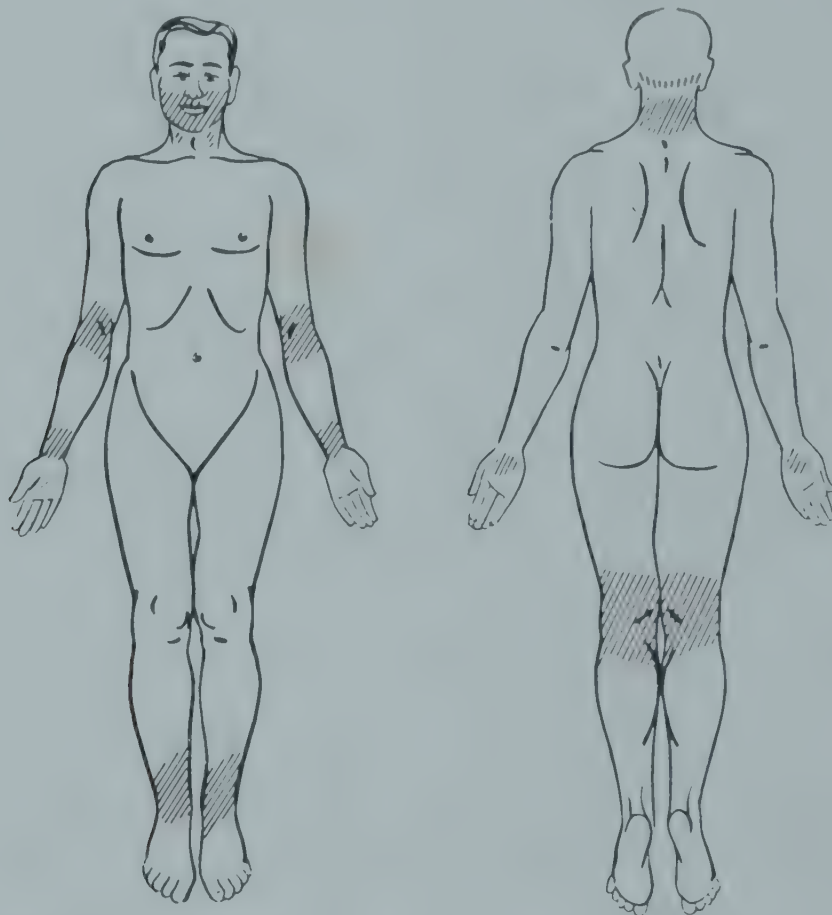


FIG. 3.—Areas of highest average pH on the bodies of subjects with Besnier's prurigo. Glass-electrode measurements. From Anderson (1). (Reproduced by permission of the author and H. K. Lewis & Company, Ltd.)

titis and in exfoliative dermatitis, the values shift noticeably toward the alkaline side (34, 55, 31). Wherever there is admixture of serous fluid, the reaction is neutral or slightly alkaline. With the healing of the process, the original acid pH returns (31). A slightly increased, but still frankly acid, pH was found in alkali-irritated skin by Lustig-Perutz (34) and in tinea capitis by Herrmann *et al.* (19).

The content of most cutaneous blisters is

vesicle content with litmus paper could be used as a simple clinical diagnostic test. Because of the acidity of the vesicle content in dysidrosis, he assumed that these vesicles are formed by sweat retention. Pereiro Miguens (45) found that in hyphomycetic infections the pH of the interdigital spaces of the toes is strongly acid, as contrasted with the pH in monilial infections. In the latter the reaction was found to be less acid and closer to normal.

V. BUFFERING CAPACITY

When dilute acids or alkalies are brought in contact with the skin surface, the induced change in the pH is only temporary, and the original acid reaction is rapidly restored. In other words, the skin surface acts as a buffer system which, within a certain range, maintains its original pH. This phenomenon has been known since 1892, when Heuss (22) observed the discoloration of a filter paper soaked with alkaline red phenolphthalein solution when brought in contact with the skin. With modern indicators the buffering capacity of the skin was studied in detail, first, by Sharlit and Scheer (59), who found that buffering of acids is more complete than that of alkalies.

In modern times Burckhardt (8-11) dealt with the so-called "neutralization capacity" of the skin surface by using methods which consist essentially of measuring the time period needed for neutralization of certain concentrations and amounts of acids and alkalies. Studying individual differences, Burckhardt's first principal result was that there is a correlation between the alkali-neutralizing capacity and the alkali tolerance of the skin: individuals whose skin is rather sluggish in neutralizing alkalies are more likely to suffer damage from contact with alkalies. Recently, Burckhardt (11) found this to be true also for acids: good acid neutralization accompanies good acid resistance of the skin. He found that neutralization capacity and resistance both to acids and to alkalies usually go hand in hand in the same individuals. Persons with industrial eczema and other contact dermatitides are often unusually sensitive to both acids and alkalies, whereas persons with "endogenous eczema" (atopic individuals) have normal sensitivity to acids and alkalies. Vermeer (68), working with a different method, found that many patients with occupational eczema neutralize alkali of different concentrations in a different pattern than do normal patients. Burckhardt (10) also emphasized that nonspecific damage by

alkali deriving from poor neutralization ability may pave the way to all kinds of contact dermatitides and sensitizations. That this actually happens was shown by Haxthausen (18).

Numerous authors have confirmed Burckhardt's observation on correlations between poor alkali neutralization and alkali sensitivity. Observations on acid neutralization, however, are contradictory. Schuppli (58) found fairly uniform responses in normal subjects but greater resistance and faster neutralization in "eczematous" patients. He attributed that to a higher alkalinity of the surface in the alkali-sensitive eczematous patients. He found that acid resistance is greater when the "eczema" is worsening and that it approaches the normal pattern when the "eczema" is clearing. According to his findings, patients with eczema are more sensitive to alkalies, but more resistant to acids, than normals. Schuppli (58) could not demonstrate any change in the neutralization pattern after oral administration of ammonium chloride or sodium bicarbonate, and he found no relation between the acid neutralization and the alkali reserve of the blood or the pH of the urine.

Schmidt (54) was able to measure continuously the acid binding of the skin with great accuracy. He found that although there are characteristic individual differences, the pattern is fairly uniform. Individual differences might have been partly caused by differences in thickness of the horny layer. He did not investigate correlations between acid neutralization and tendency to skin diseases.

In a recent careful study Klauder and Gross (29) closely followed the neutralization on dorsa and palms of hands after the use of soaps and detergents and discussed the significance of their findings for the development of occupational dermatoses.

The nature of the buffering system of the skin surface has been discussed many times. Most authors agree that water-soluble con-

stituents of sweat deposited in the horny layer play a predominant role. The most impressive evidence for this was presented by Robert and Jaddou (48) and by Wöhnlich (70, 71), who demonstrated that atropinization slows down considerably the speed of neutralization, and by Robert and Jaddou (48) and by Vermeer (68), who found that profuse sweating initially greatly accelerates the neutralization of alkalis (see also Burckhardt [10]). Conversely, if the skin is soaked with water, whereby water-soluble substances are removed from the surface and from within the horny layer, the neutralization capacity is greatly reduced (69). It must be due to the reaction with water-soluble substances that by far the most intense neutralization is observed in the first 5 minutes when the skin is in contact with acid or alkaline solutions (69, 10, 54).

In addition to sweat constituents, the keratin of the stratum corneum was also thought to be able to buffer the reaction of the skin surface, keratin being an amphoteric protein with the ability to neutralize acids and alkalis (50, 59, 47, 8–11, 57, 25, 32). Actually, scales scraped from normal skin do bind just a little alkali in vitro (33).¹ The experiments of Vermeer *et al.* (69) seem to indicate that the buffering capacity of keratin in the horny layer is very limited and plays a minor role, if any. If it were operating in alkali neutralization, it should bind the cation of the alkali and make it disappear from the solution which is in contact with the skin. According to Vermeer, this is not the case (69). Still, a modifying action of keratin is assumed (32). Of course, without an intact keratin layer, neither a physiological surface pH nor normal neutralization capacity can be maintained (2). It was found that the thicker the horny layer, the better the buffering (10, 69); but this might be due to a greater storage of water-soluble buffers within a thicker horny layer.

Vermeer (69) puts great emphasis on his

¹ For detailed studies on the acid and alkali binding properties of keratin see Steinhardt and Zaiser (61).

conclusion that adsorption or other colloid-chemical processes do not play any role in the neutralization phenomenon. All that could be demonstrated in his experiments was formation of water from H^+ and OH^- ions, as is the case in any acid-alkali reaction.

Among the sweat constituents, the lactic acid/lactate system was considered to be primarily responsible for the buffering capacity. According to Fishberg and Bierman (16), this system is a powerful buffer, around pH 4.0–4.5, in which half the lactate is ionized and half is present in the form of non-ionized lactic acid. Recently, however, Vermeer *et al.* (69) showed that after alkaline solutions had been in contact with the defatted human skin surface, they contain amino acids; and these authors believed that the principal factors in buffering the surface are the amphoteric amino acids which are secreted by the sweat glands. According to Jacobi (25), if the amino groups of amino acids and proteins are bound by formaldehyde or by nitrous acid, both the acidity of the surface and the alkali-neutralization capacity increase. This would indicate the role of the carboxylic groups of amino acids. Piper (47) found good buffering capacity from pH 4 to pH 8, with a maximum at pH 6.5, which again speaks more for the decisive role of amino acids than of lactic acid. He calculated in one case the dissociation constant of the acid mixture as delivered by the skin to be $K = 2.95 \times 10^{-5}$, which is in the range of constants of carboxylic acids in general.

Piper (47) dealt in great detail with the possible role of carbon dioxide and came to the conclusion that the neutralization is partly due to the diffusion of CO_2 from the inside to the surface (p. 580). If a tube filled with a fixed alkali solution is placed on the skin, after a while there is no more uptake of amphoteric substances from the skin surface proper, but the diffusion of CO_2 increases. Vermeer *et al.* (69) thought to eliminate the role of CO_2 by adding excess hydrochloric acid to the solution which had been in contact with the skin and boiling this solution before titrating. In this way they found that

the carbon dioxide/carbonate system does not play any role in surface buffering. However, Vermeer (69) paid most attention to what was happening in the first few minutes of the neutralization process, whereas Piper (47) followed the events up to 1 hour. Piper's own conclusion was that, for $\frac{1}{2}$ hour, alkalis are neutralized on the skin by the skin's own amphoteric substances but that in the second half-hour diffusing carbon dioxide takes over. Piper's observation of increased CO_2 diffusion when a 0.02 N NaOH solution remains in contact with the skin for 1 hour might, of course, be due to a nonphysiological loosening of the "diffusion membrane" in the skin.

It was among Burckhardt's earliest observations (8, 9) that if the 5-minute alkali-neutralization experiment is repeated subsequently several times in the same area, the later neutralization times are longer than the earlier ones, but finally become constant. His interpretation was that in the beginning the fixed acids on the skin surface bind the alkali, but, later on, it is the steadily diffusing carbon dioxide which takes care of the neutralization. This view was accepted by Szakall (62, 63) and by Neuhaus (43), who experimented with conventional soaps and neutral detergents. Szakall found that soaps, by defatting the surface, facilitate carbon dioxide diffusion, and, therefore, the restoration of the original pH is usually rapid. A single soap washing removes about 50 per cent of the total surface lipids. A most important factor in the soap effect, however, is the state of hydration of the horny layer after rinsing, because the hydrated horny layer retains carbon dioxide and slows down its diffusion. Thereby moderate hydration becomes desirable, promoting the maintenance of an acid reaction.

Washing even with water alone causes moderate hydration, with a drop in pH from 6.3 to 5.3 in 30 minutes (63). The degree of swelling or shriveling of the horny layer after washing with water and soap depends on the quality of the soap and on the atmospheric conditions. In cold weather extreme dehydration of the horny layer is

certainly not desirable, as it may lead to cracking. In warm weather, when the keratin is inclined to swell, soaps with greater dehydrating effect can be used (63).

Neuhaus (43) and Szakall (62, 63), from their experiments with soaps, came to the conclusion that constant penetration of carbon dioxide to the skin surface from below promotes alkali neutralization on the surface. This diffusion increases when the surface is defatted (69, 43).

The question of whether the normal free fatty acids on the skin surface take any part in buffering was studied by Lincke (32). He found that sebum has no acid-buffering capacity at all, and an extremely weak buffering effect on alkalies, the maximum effect being at pH 9, where $3-5 \times 10^{-4}$ mol alkali is neutralized by 1 gm. of fat. This would amount to neutralization of 12-20 mg. of NaOH by 1 gm. of sebum. That much buffering is indeed negligible.

Most neutralization experiments have been carried out after cleansing of the skin with fat solvents, which remove most of the fatty acids; and invariably neutralization proceeds faster when fat is removed. Vermeer (69) and Neuhaus (43) believe that greater diffusion of carbon dioxide in defatted skin may contribute to this effect. Be that as it may, the fatty layer no doubt plays a role in neutralization, in so far as it protects to some degree against contact with aqueous solutions of acids and alkalies by simply slowing down their penetration (16, 15, 67).

M. Schmid (53) found a considerable shift toward the alkaline side of the surface pH and of the pH of sweat in cases of eczema and seborrheic dermatitis. There was a diminished alkali neutralization but increased acid neutralization capacity in the same diseases. In seborrheic dermatitis the high pH values persisted after the eruption cleared. Some therapeutic effects were observed in eczema after internal administration of hydrochloric acid; at the same time, surface pH and acid neutralization became normal. The author was able to modify surface pH also by local application of vari-

ous ointment bases for several hours. It was pointed out that because the isoelectric point of keratin is at pH 5, any increase of pH above this value promotes the hydration (swelling) of keratin.

The recent findings of Anderson (1) and of Schmid (53) on abnormal alkalinity of the surface in seborrheic dermatitis and in atopic dermatitis are most remarkable and deserve further study.

BIBLIOGRAPHY

1. ANDERSON, D. S. The acid-base balance of the skin, *Brit. J. Dermat.*, **63**:283-96, 1951.
2. ARNOLD, L., and BART, A. The self-disinfecting power of skin, *Am. J. Hyg.*, **19**:217-28, 1934.
3. BERGEIM, O., and CORNBLEET, T. Acidity of the scalp; nature and possible relation to seborrhea, *Arch. Dermat. & Syph.*, **56**:448-51, 1947.
4. BLANK, I. H. Measurement of pH of the skin surface. I. Technique, *J. Invest. Dermat.*, **2**:67-74, 1939.
5. ———. Measurement of pH of the skin surface. II. pH of the exposed surfaces of adults with no apparent skin lesions, *ibid.*, pp. 75-79.
6. Measurement of pH of the skin surface. III. Measurements on the arms of children with no apparent skin lesions, *ibid.*, pp. 231-34.
7. ———. Measurement of pH of the skin surface. IV. Daily variations for adult females with no apparent skin lesions, *ibid.*, pp. 235-42.
8. BURCKHARDT, W. Beiträge zur Ekzemfrage. II. Die Rolle des Alkali in der Pathogenese des Ekzems speziell des Gewerbeekzems, *Arch. f. Dermat. u. Syph.*, **173**:155-67, 1935.
9. ———. Beiträge zur Ekzemfrage. III. Die Rolle der Alkalischädigung der Haut bei der experimentellen Sensibilisierung gegen Nickel, *ibid.*, pp. 262-66.
10. ———. Neuere Untersuchungen über die Alkaliempfindlichkeit der Haut, *Dermatologica*, **94**:73-96, 1947.
11. BURCKHARDT, W., and BÄUMLE, W. Die Beziehungen der Säureempfindlichkeit zur Alkaliempfindlichkeit der Haut, *Dermatologica*, **102**:294-300, 1951.
12. CZETSCH-LINDENWALD, H. VON. Measurement of skin pH, *Fette u. Seifen*, **47**:401-4, 1940. Quoted in *Chem. Abstr.*, **35**:8205, 1941.
13. DILL, D. B.; HALL, F. G.; and EDWARDS, H. T. Changes in the composition of sweat during acclimatization to heat, *Am. J. Physiol.*, **123**:412-19, 1938.
14. DRAIZE, J. H. The determination of the pH of the skin of man and common laboratory animals, *J. Invest. Dermat.*, **5**:77-85, 1942.
15. DÜNNER, M. Der Einfluss des Hauttalges auf die Alkaliabwehr der Haut, *Dermatologica*, **101**:17-28, 1950.
16. FISHBERG, E. H., and BIERMAN, W. Acid-base balance in sweat, *J. Biol. Chem.*, **97**:433-41, 1932.
17. HARDT, L. L., and PALMER, A. Studies in intermittent heat sweats; the chlorides and the acid-base balance, *Am. J. Digest. Dis. & Nutrition*, **4**:489-92, 1937.
18. HAXTHAUSEN, H. Verwandtschaftsreaktion bei Nickel- und Kobalt-Allergie der Haut, *Arch. f. Dermat. u. Syph.*, **174**:17-21, 1936.
19. HERRMANN, F.; BEHRENDT, H.; and KARP, F. L. On the acidity of the surface of the scalp and other areas of the skin in children, *J. Invest. Dermat.*, **7**:215-26, 1946.
20. HERRMANN, F., and FÜRST, K. Über die Schweißsekretion und ihre Bedeutung bei Dermatosen, *Dermat. Wchnschr.*, **88**:397-409, 1929.
21. HERRMANN, F.; PROSE, P. H.; and SULZBERGER, M. B. Studies on sweating. V. Studies of quantity and distribution of thermogenic sweat delivery to the skin, *J. Invest. Dermat.*, **18**:71-86, 1952.
22. HEUSS, E. Die Reaktion des Schweißes beim gesunden Menschen, *Monatsh. f. prakt. Dermat.*, **14**:343, 400, and 501, 1892.
23. HIER, S. W.; CORNBLEET, T.; and BERGEIM, O. The amino acids of human sweat, *J. Biol. Chem.*, **166**:327-33, 1946.
24. HOPF, G. Chemisch-physikalische Untersuchungen über menschlichen Schweiß, *Arch. f. Dermat. u. Syph.*, **171**:301-12, 1935.
25. JACOBI, O. Über die Reaktionsfähigkeit und das Neutralisationsvermögen der lebenden menschlichen Haut, *Dermat. Wchnschr.*, **115**:733-41, 1942.
26. KARITZKY, B. Vegetative Belastung und Schweißfunktion, *Ztschr. f. d. ges. inn. Med.*, **2**:555-61, 1947.
27. KARITZKY, B.; RAABE, S.; and UGI, I.

- Wasserstoffzahl und Säuregehalt des Schweißes bei chirurgischen Kranken, Arch. f. klin. Chir., **263**:246-60, 1949.
28. KITTSTEINER, C. Sekretion, Kochsalzgehalt und Reaktion des Schweißes, Arch. f. Hyg., **73**:275-306, 1910-11.
 29. KLAUDER, J. V., and GROSS, B. A. Actual causes of certain occupational dermatoses. III. A further study with special reference to effect of alkali on the skin, effect of soap on pH of skin, modern cutaneous detergents, Arch. Dermat. & Syph., **63**:1-23, 1951.
 30. KORKISCH, H. Die H-Ionenkonzentration im Pferdeschweisse, Arch. f. d. ges. Physiol., **213**:539-43, 1926.
 31. LEVIN, O. L., and SILVERS, S. H. The reaction of the skin and its secretions in eczema. The hydrogen ion concentration of the skin surface in eczema, Arch. Dermat. & Syph., **25**:825-34, 1932.
 32. LINCKE, H. Beiträge zur Chemie und Biologie des Hautoberflächenfetts, Arch. f. Dermat. u. Syph., **188**:453-81, 1949.
 33. LUSTIG, B., and PERUTZ, A. Über ein einfaches Verfahren zur Bestimmung der Wasserstoffionenkonzentration der normalen menschlichen Hautoberfläche, Arch. f. Dermat. u. Syph., **162**:129-34, 1930.
 34. ———. Über die Hydrogenionenkonzentration der Hautoberfläche und ihre Regulation bei Dermatosen, *ibid.*, **163**:18-29, 1931.
 35. MARCHIONINI, A. Physikalisch-chemische Untersuchungen an menschlicher Haut, Klin. Wchnschr., **6**:284-85, 1928.
 36. ———. Physikalisch-chemische Untersuchungen über ekkrinen und apokrinen Schweiß, Schweiz. med. Wchnschr., **58**:1055-57, 1072, 1928.
 37. ———. Die Wasserstoff-Ionenkonzentration des Schweißes, Klin. Wchnschr., **8**:924-26, 1929.
 38. ———. Untersuchungen über die Wasserstoffionenkonzentration der Haut, Arch. f. Dermat. u. Syph., **158**:290-333, 1929.
 39. ———. Säurebehandlung intertriginöser Epidermophytien, Dermat. Ztschr., **56**:248-56, 1929.
 40. ———. Zur Pathogenese und Differentialdiagnose dyshidrotischer und dyshidrosiformer Bläschenerkrankungen der Hände und Füße, *ibid.*, **58**:222-35, 1930.
 41. MARCHIONINI, A., and CERUTTI, P. Physikalisch-chemische Untersuchungen zur Pathogenese der Bromhidrosis pedum, Arch. f. Dermat. u. Syph., **166**:354-62, 1932.
 42. MARCHIONINI, A., and HAUSKNECHT, W. Säuremantel der Haut und Bakterienabwehr; die regionäre Verschiedenheit der Wasserstoffionenkonzentration der Hautoberfläche, Klin. Wchnschr., **17**:663-66, 1938.
 43. NEUHAUS, H. Fettgehalt und Alkalineutralisationsfähigkeit der Haut unter Anwendung alkalifreier Waschmittel, Arch. f. Dermat. u. Syph., **190**:57-66, 1950.
 44. OTTENSTEIN, B. Beitrag zur Chemie des Schweißes, Arch. f. Dermat. u. Syph., **191**:116-22, 1950.
 45. PEREIRO MIGUENS, M. El pH y la flora de los pequeños pliegues, Actas dermo-sif., **41**:607-32, 1950. Quoted in Excerpta med., Sec. XIII, **5**:6, 1951.
 46. PEUKERT, L. Einfluss der Titrationsalkalität von Reinigungsmitteln auf den pH-Wert der menschlichen Haut, Arch. f. Dermat. u. Syph., **181**:417-24, 1940.
 47. PIPER, H. G. Das Neutralisationsvermögen der Haut gegenüber Laugen und seine Beziehung zur Kohlensäureabgabe, Arch. f. Dermat. u. Syph., **183**:591-647, 1943.
 48. ROBERT, P., and JADDOU, J. Untersuchungen über den Einfluss der Schweißsekretion auf die Alkalineutralisationsfähigkeit der Haut, Dermatologica, **86**:72-78, 1942.
 49. ROTHMAN, S. Haut und Hautanhänge. In: OPPENHEIMER, Handb. d. Biochem. d. Menschen u. d. Tiere, suppl. vol., **2**:157-79. 2d ed. Jena: G. Fischer, 1934.
 50. ROTHMAN, S., and SCHAAF, F. Chemie der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1/2**:161-377. Berlin: J. Springer, 1929.
 51. SCHADE, H., and MARCHIONINI, A. Der Säuremantel der Haut (nach Gaskettenmessungen), Klin. Wchnschr., **7**:12-14, 1928.
 52. ———. Zur physikalischen Chemie der Hautoberfläche, Arch. f. Dermat. u. Syph., **154**:690-716, 1928.
 53. SCHMID, M. Vergleichende Untersuchungen über die Säure-Basen-Verhältnisse auf der Haut, Dermatologica, **104**:367-91, 1952.
 54. SCHMIDT, P. W. Über die Beeinflussung der Wasserstoffionenkonzentration der Hautoberfläche durch Säuren. Betrachtungen über die Funktionen des "Säuremantels," Arch. f. Dermat. u. Syph., **182**:102-26, 1941.

55. SCHOLTZ, W. Über saure Behandlung von Hautkrankheiten, *Klin. Wchnschr.*, **9**: 1670-72, 1930.
56. SCHULTZ, F. N. Hautabscheidung und Tränen. In: OPPENHEIMER, Handb. d. Biochem. d. Menschen u. d. Tiere, **5**:385-418. Jena: G. Fischer, 1925.
57. SCHULZE, W. Untersuchungen über die Alkaliempfindlichkeit, das Alkalineutralisationsvermögen und die Kohlensäureabgabe der Haut, *Arch. f. Dermat. u. Syph.*, **185**: 93-161, 1943.
58. SCHUPPLI, R. Untersuchungen über das Säure-Neutralisationsvermögen der Haut, *Dermatologica*, **98**:295-301, 1949.
59. SHARLIT, H., and SHEER, M. The hydrogen ion concentration of the surface on the healthy intact skin, *Arch. Dermat. & Syph.*, **7**:592-98, 1923.
60. SHELLEY, W. B., and HURLEY, H. J. The physiology of the human axillary apocrine sweat gland, *J. Invest. Dermat.*, **20**:285-97, 1953.
61. STEINHARDT, J., and ZAISER, E. M. Combination of wool protein with cations and hydroxyl ions, *J. Biol. Chem.*, **183**:789-802, 1950.
62. SZAKALL, A. Über die Physiologie der obersten Hautschichten und ihre Bedeutung für die Alkaliresistenz, *Arbeitsphysiol.*, **11**: 436-52, 1941.
63. ———. Die Veränderungen der obersten Hautschichten durch den Dauergebrauch einiger Handwaschmittel, *ibid.*, **13**:49-56, 1943.
64. TALBERT, G. A. Effect of work and heat on the hydrogen ion concentration of the sweat, *Am. J. Physiol.*, **50**:433-42, 1919-20.
65. ———. Further studies on the hydrogen ion concentration of human sweat, *ibid.*, **61**: 493-500, 1922.
66. THURMON, F. M., and OTTENSTEIN, B. Studies on the chemistry of human perspiration with special reference to its lactic acid content, *J. Invest. Dermat.*, **18**:333-39, 1952.
67. VERMEER, D. J. H. The effect of sebum on the neutralization of alkali, *Nederl. tijdschr. v. geneesk.*, **94**:1530-31, 1950.
68. ———. Method for determining neutralization of alkali by skin. Quoted in *Yearbook Dermat. & Syph.*, p. 415, 1951.
69. VERMEER, D. J. H.; JONG, J. D. DE; and LENSTRA, J. B. The significance of amino acids for the neutralization by the skin, *Dermatologica*, **103**:1-18, 1951.
70. WOHNLICH, H. I. Der Einfluss der Schweisssekretion auf die Alkalineutralisationskraft der Haut, *Arch. f. Dermat. u. Syph.*, **187**: 377-82, 1948.
71. ———. II. Ein weiterer Beitrag zum Problem der Alkalineutralisationsfähigkeit der Haut, *ibid.*, pp. 383-91.

CHAPTER 9

Insensible Water Loss

I. TRANSEPIDERMAL WATER LOSS	233
II. TOTAL INSENSIBLE WATER LOSS FROM THE SKIN	236
III. OUTWARD PERMEABILITY OF THE EPIDERMIS TO WATER	239

I. TRANSEPIDERMAL WATER LOSS

AT ALL environmental temperatures there is a continuous evaporation of water from the respiratory passages and from the skin surface. This water is invisible, and therefore the process has been called "insensible water loss." The dermal part was called "insensible perspiration," to contrast it with visible sweat secretion.

"Insensible perspiration" has two separate mechanisms. There is an insensible sweat secretion at low temperatures: the droplets are so small and evaporate so fast that they cannot be seen with the naked eye (p. 156). They can be seen and/or visualized by different staining methods when observed through a dissecting microscope (21-25, 49, 33, 42). This part of the insensible water loss can be eliminated by atropinization. However, after atropinization, a considerable fraction of the insensible water loss still continues to occur. Therefore, it is concluded that, in addition to insensible sweat secretion, there is a continuous water loss through the epidermis. This part cannot be made visible, even microscopically, possibly because the water has been transformed into vapor before it has reached the surface. It was proposed that this part be called "true insensible perspiration" (8), but the expression "transepidermal water loss," indicating the nature of the process, is probably to be preferred.

Details of why and how this water "ascends" in the epidermis and where it transforms into vapor are not known. Under normal conditions the water content and vapor pressure of the epidermis are higher than those of the surrounding air (41), and one would expect loss of water to the outside on purely physical grounds. But, with increasing atmospheric humidity, the insensible water loss does not decrease in a linear fashion or does not decrease at all (37, 49, 58), or, at least, it is very difficult to demonstrate the effect of atmospheric moisture (41). Also there is no clear influence of environmental or skin surface temperature (44, 4, 20, 61).¹ Therefore it is improbable that transepidermal water loss is a purely physical diffusion process.

Pure arterial hyperemia, such as is elicited, for instance, by inhalation of amyl nitrite (37) or by alcohol intake (9), seems in some cases to increase insensible water loss through the skin, but this is not regularly observed (37, 20). In acute inflammatory processes, with increased blood flow to the skin, increased capillary pressure, and

1. Pinson (41) found that after blockade of the sweat glands the water loss is doubled when the skin temperature is increased by 10° C., which would indicate that the process is purely physical in nature. However, he worked on skin areas which were treated electrophoretically with formalin, and such skin cannot be regarded as normal.

increased skin temperature, one expects the water content and vapor pressure of the epidermis to increase if physical laws prevail; and yet the transepidermal water loss is not only not increased but mostly decreased, particularly if epidermal damage is involved (37, 27, 45). Likewise, water-vapor loss is unchanged or decreased over edematous skin (19), on pitting edema (49), as well as over histamine wheals (38, 39, 41).

On the basis of extensive studies Moog (38, 39) came to the conclusion that the transepidermal water transport is a "vital" function of the epidermal cells. He finds that formalin, which stops sweat secretion, increases transepidermal water loss; and he interprets this finding as being due to the "stimulation" of epidermal cells. He also finds that with the circulation arrested by death, insensible water loss does not stop abruptly but ceases very gradually, corresponding with the gradually decreasing metabolism and slowly ensuing death of epidermal cells. Similarly, by complete arrest of circulation, Kuno and Ikeuchi (28) could suppress insensible water loss by only about 30 per cent (from 4.5 to 3.1 mg.).

My idea that transepidermal water loss is closely connected with the keratinization process was first mentioned in 1929 (49). Keratinization is associated with dehydration of the cellular protoplasm (p. 374). The "hydration" water, when liberated, is evaporated either from the surface or from between the horny lamellae. This theory was supported experimentally by the demonstration of increased water loss from lesions in which the keratinization process is accelerated because of increased proliferation of rete cells (46). Further work to prove this theory was done in 1945, when Felsher and Rothman (12) demonstrated that in lesions of psoriasis and in exfoliative dermatitis—conditions in which keratinization is greatly accelerated—insensible perspiration is increased three to ten times. While in these conditions, in addition to accelerated keratinization, increased permeability to water possibly participated in enhancing transepidermal water loss, such an additional factor

need not be assumed in the state of postinflammatory scaling. In ultraviolet erythema there was no increase (45) or, at best, only a moderate increase (12) of insensible water loss. But 4–9 days after irradiation, when the erythema subsided and scaling began, the insensible perspiration rose to over twice the normal figures (12).

Results obtained on transepidermal water loss after repeated galvanic introduction of a 5 per cent formaldehyde solution from the anode according to the method of Kuno and Kashiwabara (29), as used by Pinson (41), do not necessarily reflect a physiological situation. It was stated that on further repetition of this procedure the skin becomes scaly, dry, and irritated (41); and it can be assumed that a subclinical "irritation" occurs long before these gross changes appear. I am inclined to attribute the increase of transepidermal water loss after formalin treatment (37, 41) to an intensified keratinization, such as always occurs in subacute inflammatory processes. Altogether, the "vital processes of the epidermis," as postulated by Moog (37), can be well explained by changes in the rate of keratinization. The assumption that transepidermal water loss is, in part at least, a function of the keratinization process was substantially supported by Vasti (58), when she showed that the water evaporating from the surface behaves not like free water but like hydration water, since it has a considerably lower vapor tension than free water.

It is generally agreed that water lost through the epidermis, as contrasted with sweat, does not contain salts or other solutes. It is indeed remarkable how little chloride is found on the skin surface after a person has not bathed and not visibly sweat for 1 week. Hancock *et al.* (14) recovered from the bathing water of such individuals only 83 mg. chloride. Because there was almost as much potassium in this water (77 mg.), it was concluded that this material originates from sebaceous and keratinous debris (p. 212).

Under normal conditions sweat and transepidermally lost water mix with each

other in the horny layer and on the surface, and therefore they cannot be separated. The situation becomes even more complicated if one accepts the claim that there is a free interchange of water and solutes between epidermis and the intraepidermal portion of the sweat duct (p. 193). However, Vasti (58) was able to demonstrate continuous water loss in most of her experiments without finding any traces of chloride on the skin surface.

Whitehouse, Hancock, and Haldane (59) maintained that loss of water through the epidermis is due to osmotic forces. According to this view, the skin separating the blood plasma from the external surface behaves like a semipermeable membrane which is slowly permeable to water but practically impermeable to inorganic salts. The difficulties in interpreting transepidermal water loss as a purely physical process have been mentioned. The particular concept of Haldane has the additional difficulty that water should not migrate outward at all, if osmotic equilibrium is to be approached, because the aqueous film on the skin surface is said to be hypotonic as compared with the plasma. Nevertheless, Whitehouse *et al.* (59) found, in conformity with their theory, that in a distilled water bath at 33°–34° C. there is no outward loss of water through the skin. There may even be some gain in body weight because water moves inward by osmosis. If salt is added to the bath, cutaneous water loss sets in and increases with increasing salt concentration. These data were confirmed by Trolle (55).

There has been much speculation about the routes of migration of water in the epidermis. Seventy years ago Unna (56) demonstrated a system of minute canals in the dermis which had their origin at the apices of the papillae, spread in radial fashion toward the surface layers, and, after bending downward again, finally ended in the ducts of the sweat glands. He assumed that water moves through this canal system, and he was the first to state that the appearance of water at the sweat-gland orifices does not necessarily mean that this water is the product of sweat glands, because there is free in-

terchange of fluid between the epidermis and the epidermal portion of the sweat duct. Melczer (36), on the basis of experiments with aqueous urea infusions, came to similar conclusions. He found that there is an exchange of materials between the epidermal portion of the duct and the canal system of the epidermis, even if the exchange is not so free as Unna thought it to be. He also assumed that water diffuses from the cutaneous vessels into the epidermis in a liquid state and evaporates in the upper third of the horny layer. According to Frieboes (13), the passage of water through the epidermis is effected largely by the epidermal fiber system, "which behaves, to put it drastically, like a hydrophilic tridimensional network with its lower free end hanging down into a water pool."

If the theory that transepidermal water loss is due to the dehydration of keratinizing cells is correct, no route by which water migrates upward must be assumed. Water "migrates" upward only in so far as it ascends as intracellular water with the rete cells and is liberated only when the cells keratinize. Frieboes' concept fits in, in so far as the keratin fibers are drier than the intracellular fibers in the rete cells.

Persons with the anidrotic type of multiple ectodermal dysplasia are ideal subjects for the physiological study of transepidermal water loss. This condition is a genetic anomaly, in which, in addition to developmental disturbances of hair, teeth, and other ectodermal structures, the eccrine glands are entirely or almost entirely (11) absent. In these persons the water loss through the skin is practically pure transepidermal loss.

Physiological studies on such individuals were carried out by Loewy and Wechselmann (34), Richardson (43), Sunderman (54), Felsher (11), Upshaw and Montgomery (57), and others.

At rest and at 24°–25° C. air temperature, these persons feel perfectly well and eliminate normal quantities of water through their skin. Their evaporative heat loss participates in the total heat loss in an entirely normal fashion (43). But if their metabolism

is increased by exercise beyond a certain degree or the air temperature is raised above the comfortable zone, they are unable to respond to the increased heat production with a corresponding heat loss because of their inability to sweat. Their body temperature rises rapidly to fever temperatures, and they become hyperpneic. In some cases polyuria was observed after heating; this is either because the hyperpnea leads to alkalosis and alkalosis to diuresis or because water is liberated from the tissues by the heat and, as it cannot be secreted by sweat glands, escapes through the kidneys (54).

The fact that at comfortable temperatures cutaneous insensible water loss in persons without sweat glands has always been found normal leads to the conclusion that, in normal persons, there is no sweat-gland function at these temperatures. However, in our own experience there always is a difference in insensible water loss before and after atropinization in normal individuals (45), whereas such a difference is not found in anidrotic ectodermal dysplasia (11).

There seems to be no mechanism in these persons which would compensate to any appreciable degree for the absence of sweat

glands. Increased evaporation from the respiratory passages produced by hyperpnea does not inhibit prompt elevation of body temperature on heating. Richardson (43) found that heat loss by radiation and convection is not greater than in normals. Nevertheless, it is remarkable that, on heating, the skin temperature of these persons is greatly increased as compared with normals: Sunderman (54) measured, in the hot room, 102°8 and 104°0 F. skin temperatures, respectively, in two patients, as compared with 97°8 F. in normal controls. The respective body temperatures were 101°4 and 102°0 F. in the patient and 98°6 and 98°4 F. in the normal controls. Obviously, the skin is not cooled because of absence of evaporating sweat, and it is hard to believe that heat loss by radiation would not increase under such circumstances.

Apocrine sweat glands are fully developed and function normally in these individuals (11) (Fig. 1). It is not known, however, whether they measurably counteract the hyperthermia. The hyperthermia can be counteracted by sprinkling the body surface with water. The sprinkling functions as a system of "artificial sweat glands" (43).

II. TOTAL INSENSIBLE WATER LOSS FROM THE SKIN

In most experiments concerned with "insensible perspiration" of the skin, invisible sweat secretion and transepidermal water loss are not separated, but the sum of the two is measured. The ratio of the two components is extremely variable and apparently depends primarily on the mental tension of the test person on whom the measurements are made. I observed that in clinical patients the total vapor loss is greater, and a larger portion of it can be eliminated by atropinization, than in members of the staff who are used to such experimentation on their skin. In the latter group the average ratio at comfortable room temperature was found to be one-tenth sweat-gland function and nine-tenths transepidermal water loss on the trunk. On the palms and soles the participation of sweat glands is much great-

er (17). In any case, the ratio is so variable that no conclusions can ever be drawn about the participation of the two mechanisms if they are not artificially separated by atropinization (45, 18, 19, 20, 8).

The majority of authors still maintain that at low environmental temperatures sweat glands do not take part in the insensible water loss; and Kuno emphasized that microscopic proof for sweat secretion at low temperatures can be obtained only on palms and soles. However, Randall (42), with his sensitive visualization method, found periodic sweat-gland activity also on the forearm at a room temperature of between 20° and 27° C. My finding that there is always a difference in values on the trunk before and after atropinization, together with similar findings of Jores (18, 19, 20) and of Chro-



FIG. 1.—Function of apocrine glands in ectodermal dysplasia. View showing confluence of sweat droplets in areas of apocrine glands (axillae, upper part of chest, and perimammillary regions). Sweat visualized by Minor's method as dark patches. From Felsher (11). (Reproduced by permission of the author and the American Medical Association.)

metzka and Schweder (8), leaves little doubt that there is an "insensible" sweat secretion all over the body surface, even if it is sometimes exceedingly small. The periodicity of water-vapor delivery at low temperatures (42, 40, 1, 2) also indicates participation of sweat glands.

The total insensible water loss through skin and lungs is constant under basal conditions and amounts to 200 gm/sq meter/24 hr. About 60 per cent of this amount is cutaneous water loss (18, 3, 27, 59). The total cutaneous water loss in adult men at rest and without visible sweating was estimated to be between 500 and 700 gm. daily (49, 27). The cutaneous part shows a definite decline with age (20) and is slightly, but distinctly, lower in women than in men (15, 18, 19, 20). However, this sex difference was not established after atropinization, and thus the sex difference may be due to the difference in sweating ability (p. 160). According to Jores (18), the total insensible loss is increased at night, apparently proportionately to the depth of sleep. This, again, could be caused by greater sweat-gland activity at night, which is related to increased skin temperature in bed (50).

The total insensible loss from skin and lungs shows a linear relationship to the basal metabolic rate. This fundamental discovery of Benedict (4, 3) has often been confirmed (18, 19, 20, 30, 35). This relationship exists because the total heat loss is a function of the metabolism, and the evaporative heat loss is a fairly constant fraction of the total heat loss, viz., 24 per cent, on the average (53). The total insensible water loss, including lungs, remains constant at between 24° and 30° C. (16, 61). Under nonbasal conditions, parallelism of metabolic rate and insensible water loss can be demonstrated with much greater accuracy if the test person is atropinized (18, 19, 20).

Of course, it is hard to reconcile the concept that insensible perspiration is a thermoregulatory function with the view that it has something to do with keratinization. However, it is by no means impossible that the rate of keratinization as a function of epi-

thelial proliferation closely follows the metabolic rate.

Water intake has no influence on cutaneous water loss in the adult (49, 19, 50), but it increases insensible water loss in children, whose water metabolism is more labile and in whom extrarenal water excretion is generally higher than in adults (49, 8, 16). In adults insensible perspiration also remains uninfluenced by subcutaneous or intravenous physiological fluid infusions and after administration of antidiuretic posterior pituitary preparations (8). There is, however, a diminution in extreme dehydration (19).

Retention of salt and water in renal and cardiac diseases was thought to depress insensible water loss (49) considerably, but recently negative results have been reported in cases of cardiac failure.²

In hypothyroidism, values are conspicuously low; they increase rapidly under thyroid medication. In thyrotoxicosis the total insensible perspiration is greatly increased, as well as the cutaneous water loss after atropinization (20). In diabetes, water loss is decreased (22).

It appears that gross shifts in the salt and water balance of tissues, as they can be produced in acute experiments by administration of hypertonic and hypotonic salt solutions parenterally, have a profound influence on insensible water loss, hypertonic solutions binding water and therefore decreasing, hypotonic solutions releasing water and therefore increasing, insensible loss. The same was claimed to be true for visible sweat secretion (49).

Most greasy coverings on the skin hinder transepidermal water loss (44, 20). Apparently, if the grease is miscible with water, this effect is less pronounced (51). In any case, the natural greasy film of the human skin surface does not seem to play any role: in severe postencephalitic seborrhea, Jores (20) did not find any reduction in insensible perspiration, in spite of the

2. C. J. D'Alton, R. C. Darling, and E. Shea, The insensible loss of water in congestive heart failure, *Am. J. M. Sc.*, **216**:516-22, 1948.

presence of a thick oily layer. Estimating insensible water loss in vivo under different covers, I found that a petrolatum cover decreases it by 50 per cent but zinc paste by only 20–30 per cent, because of the more porous nature of the latter (44).

Inflammatory skin diseases do not increase the transepidermal water loss (45, 38, 20), except those which involve accelerated keratinization (p. 234). Greatly decreased values were found by Jores (20), in a case of "dry generalized hyperkeratotic eczema." In ichthyosis vulgaris the values were always found to be normal (20, 46, 12). This seems to indicate that ichthyosis is not connected with an increased rate of keratinization but rather is an anomaly of the shedding process (12). Insensible perspiration is diminished over scars and sclerodermatic skin (52), but the possible role of sweat-gland atrophy in this diminution has not been excluded.

Total cutaneous insensible water loss in comfortable environments is fairly equal all over the body surface, except for the palms and soles, which, because of their more continuous and intense insensible sweating, lose the most water vapor. Ikeuchi and Kuno (17) differentiate three zones in which insensible perspiration has different levels. With the figures indicating loss of water vapor in milligrams per 20 square centimeters per 5 minutes, these zones are: zone 1, palms and soles, 5–10 mg., sometimes even 20 mg.; zone 2, forehead, neck, cheeks, dorsa of hands, 2.5–4.5 mg.; zone 3, all other parts of the body, 0.7–1.7 mg. Kuno (27) pointed out that uncovered parts lose more water than those which are clothed. Sode-man and Burch (52) find the rate of insensible perspiration in a comfortable environment to vary regionally in the following descending order: hands, feet, head, arms, legs, trunk.

III. OUTWARD PERMEABILITY OF THE EPIDERMIS TO WATER

The fact that there is transepidermal water loss leaves no doubt that water does penetrate the epidermis from the inside to the outside, even if only in small amounts. On excised skin, used as a membrane, this was demonstrated as early as 1875 (10; see also 56, 26). Recently, this whole situation was re-examined by Burch and his associates (52, 62, 6, 7), who found that diffusion of water through the skin is much slower than through any other tissue. By measuring percutaneous water loss in vivo and the outward diffusion of water through excised skin, they found the same values throughout. Furthermore, they obtained the following results: post mortem, the resistance of the skin to diffusion of water is not decreased for 14 days; after that its permeability increases about threefold, but then remains approximately at the same level for 61 days. Cantharis vesicle tops inhibit diffusion of water to the same degree as does total skin; and, on the other hand, water moves most rapidly through the remaining denuded skin after the removal of the vesicle top. In addition,

the authors abraded the "corneum" with sandpaper (*a*) lightly, (*b*) moderately, and (*c*) heavily and found that, with increasing strength of abrasion, the penetrability of the skin to water increased rapidly.

According to these authors, all their results indicated that the barrier to water diffusion from the inside to the outside is the horny layer. Histologic control of what layers are being removed by the blistering agent was done in one instance, and it was found that "the top of the blister consisted of keratinized epithelial cells." No mention was made of histological controls in the sandpapering experiments. The authors were aware of the long-established and well-evidenced view that the stratum lucidum (60) (or, more generally, the transitional layers [48]) are chiefly responsible for the inhibition of diffusion. But because they could not demonstrate the stratum lucidum in their sections taken from the epigastrium, they came to the conclusion that it is the stratum corneum which inhibits diffusion of water—a conclusion which is hard to accept

because of the great avidity with which the human horny layer takes up water from either side. The stratum lucidum can hardly be visualized with routine stains on the skin of the epigastrium, with which the authors worked. This controversial point is familiar from the discussion of what layer of the skin is the site of high electrical resistance (p. 12) and the barrier of absorption (p. 29). Time and again, the horny layer was thought to be responsible for all these barrier effects. One of the most frequently used arguments in favor of this assumption has been that these effects do not change after death and that therefore a dead layer must be responsible. Another argument has been that blister tops, which apparently consist of horny material only, have the same effect as total skin (p. 16). It has not been appreciated that nothing is known about the survival time of the cells of the stratum granulosum and stratum lucidum, although it is possible that "life" would go on longer than 24 hours or so. Even more likely than long survival is the possibility that the peculiar transitional cells of keratinization, in spite of being dead, do not change the physical properties of their surface for a long time. In any case, permeability to water increases, sometimes quite suddenly, about 14 days after death (6); and such a sudden change can be better interpreted as being due to the disorganization of previously organized structures than as due to that of dead horny lamellae. It is hard to imagine what sudden change the latter might undergo.

Experiments with abrasions and blistering agents cannot be satisfactorily evaluated until careful histologic controls with special stains for eleidin and keratohyalin accompany the physiological experimentation. Certainly, it would be hard to understand that the horny layer, which takes up water avidly because of its relative dryness and porosity, could be a barrier to water diffusion. Burch *et al.* themselves found that water diffuses through palmar and plantar skin and through toenails much more easily than through the total skin of the epigastrium, and they state that this is "difficult to

explain" (6). With the remark that "the relative proportion of alpha and beta keratin in these tissues has not been determined," the authors indicate the belief that there might be differences in the structure of horny material on palms, soles, and nails, on the one hand, and on other parts of the body, on the other. This, of course, is possible and probably true. But it is known that nail plates and palmar and plantar horny layers are not only thick but also relatively tightly woven horny structures, and, if it were true that horny structures hinder the diffusion of water, nails, palms, and soles should constitute a more formidable barrier than the thin, loose, and less keratinized horny layer of the epigastrium.

In a recent paper Berenson and Burch (5) examined the influence of environmental temperature and humidity on water penetration outward through excised skin. They found a direct, almost linear, variation with environmental temperature. These experiments were not done at constant relative humidity but at progressively decreasing relative humidities as the temperature was increased. The scattergram in Figure 2 shows that the direct variation with temperature is not striking between 15° and 25° C., which corresponds with similar findings of Richardson (43). However, in the low-temperature zone at which the dew point is approached, there is a remarkable decline in the rate of loss of water-vapor. A rapid decrease of water loss was found as the relative humidity increased when the temperature was kept constant at 39° C., the depression becoming great at the relative humidity of 90 per cent. One may speculate that at high temperatures with high relative humidity the imbibition of water by the horny layer may be caused not only by sweat retention (p. 270) but also by suppression of transepidermal water loss.

In the excised skin model of Berenson and Burch the rate of water loss from the skin of the chest, abdomen, breast, and thigh is identical. There is no difference in rate of water loss, whether the skin is normal, dehydrated, or edematous or whether it is pig-

mented or not. There are no age differences, and the water loss from the skin of Negroes is the same as that in whites. The water loss on this model is greatly suppressed by the spreading of a thin film of water-immiscible grease on the surface. Increasing air movement increases water loss only moderately (see also 44).

Berenson and Burch did not find any influence of keratin-dispersive agents, such as alkalies and sulfides, but an enormous increase of water loss after treating the excised skin with fat solvents. The interpretation of these findings was that keratin, in withholding water, acts only as a matrix in withholding lipids or lipoprotein complexes, and these latter compounds are probably the chief barrier to water loss.

The great increase in permeability of the skin when treated with lipid solvents has been known, of course, for a long time (p. 49). In my opinion the findings of Berenson and Burch can be well accounted for by the effect of lipid solvents on cell membranes. If the membranes of the cells of the stratum lucidum and stratum granulosum are burst, the relative impermeability of the skin to water is lost. This effect is tremendously increased if all the cell membranes in the epidermis are destroyed, which is the case if

excised skin is soaked in fat solvents for 1 hour. Whatever the interpretation, the recent experiments of Berenson and Burch well illustrate the salient point that the

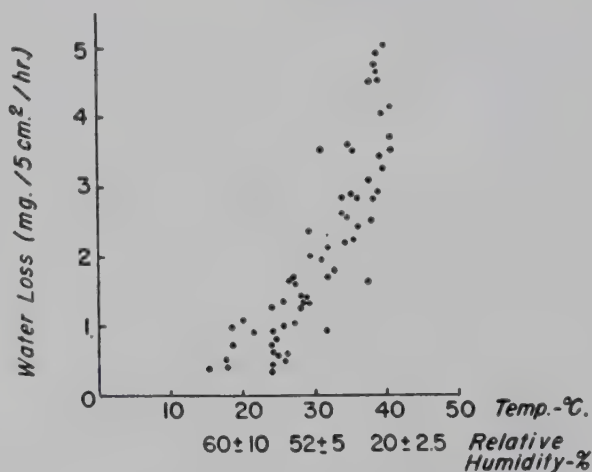


FIG. 2.—The influence of increasing temperature and decreasing relative humidity of the atmosphere upon the rates of water loss through segments of dead human skin obtained from various sites of the body (abdomen, chest, breast, and thigh). The conditions of the atmosphere are within the range of those which are frequently encountered by living man. From Berenson and Burch (5). (Reproduced by permission of the Williams & Wilkins Company.)

keratin fibers of the horny layer are not the barrier to water diffusion, inward or outward.

BIBLIOGRAPHY

1. ALBERT, R. E., and PALMES, E. D. Pulsative evaporative rate from small skin areas as measured by an infra-red gas analysis, *Federation Proc.*, **8**:1-2, 1949.
2. ———. Evaporative rate patterns from small skin areas as measured by an infra-red analyzer, *J. Appl. Physiol.*, **4**:208-14, 1951.
3. BENEDICT, C. G., and BENEDICT, F. G. The nature of the "perspiratio insensibilis," *Skandinav. Arch. f. Physiol.*, **49**:89-90, 1926.
4. BENEDICT, F. G., and ROOT, H. F. Insensible perspiration: its relation to human physiology and pathology, *Arch. Int. Med.*, **38**:1-35, 1926.
5. BERENSON, G. S., and BURCH, G. E. Studies of diffusion of water through dead human skin: the effect of different environmental states and of chemical alterations of the epidermis, *Am. J. Trop. Med.*, **31**:842-53, 1951.
6. BURCH, G. E., and WINSOR, T. Rate of insensible perspiration (diffusion of water) locally through living and dead human skin, *Arch. Int. Med.*, **74**:437-44, 1944.
7. ———. Diffusion of water through dead plantar, palmar, and dorsal human skin and through toe nails, *Arch. Dermat. & Syph.*, **53**:39-41, 1946.
8. CHROMETZKA, F., and SCHWEDER, M. Die Unbeeinflussbarkeit der Perspiratio insensibilis durch experimentelle und pharmakologische Eingriffe im Wasserhaushalt des Menschen, *Ztschr. f. d. ges. exper. Med.*, **80**:288-302, 1931.

9. EIMER, K. Untersuchungen über das Wesen der Perspiration, Arch. f. exper. Path. u. Pharmakol., **125**:150-80, 1927.
10. ERISMAN, F. Zur Physiologie der Wasserverdunstung von der Haut, Ztschr. f. Biol., **11**:1-78, 1875.
11. FELSHER, Z. Hereditary ectodermal dysplasia; report of a case, with experimental study, Arch. Dermat. & Syph., **49**:410-14, 1944.
12. FELSHER, Z., and ROTHMAN, S. The insensible perspiration of the skin in hyperkeratotic conditions, J. Invest. Dermat., **6**:271-78, 1945.
13. FRIEBOES, W. Beiträge zur Anatomie und Biologie der Haut. X. Die biologischen Funktionen der menschlichen Epidermis, Arch. f. Dermat. u. Syph., **140**:467-80, 1922.
14. HANCOCK, W.; WHITEHOUSE, A. G. R.; and HALDANE, J. S. The loss of water and salts through the skin, and corresponding physiologic adjustments, Proc. Roy. Soc. London, s.B, **105**:43-59, 1929.
15. HARDY, J. D.; MILHORAT, A. T.; and DU BOIS, E. F. Basal metabolism and heat loss of young women at temperatures from 22° to 35° C., J. Nutrition, **21**:383-404, 1941.
16. HELLER, H. Die extrarenale Wasserausscheidung beim Menschen, Ergebn. d. inn. Med. u. Kinderh., **36**:663-751, 1929.
17. IKEUCHI, K., and KUNO, Y. On the regional differences of the perspiration on the surface of the human body, J. Orient. Med., **7**:67-89 and 106-7, 1927.
18. JORES, A. Perspiratio insensibilis. I. Ztschr. f. d. ges. exper. Med., **71**:170-85, 1930.
19. ———. Perspiratio insensibilis. II. Über die Beziehungen zwischen renaler und extrarenaler Wasserausscheidung, *ibid.*, **74**:757-68, 1930.
20. ———. Perspiratio insensibilis. III, *ibid.*, **77**:734-42, 1931.
21. JÜRGENSEN, E. Mikrobeobachtungen der Schweisssekretion der Haut des Menschen unter Kontrastfärbung. I, Deutsches Arch. f. klin. Med., **144**:193-201, 1924.
22. ———. Mikrobeobachtungen der Schweisssekretion der Haut des Menschen unter Kontrastfärbung. II. Funktionsprüfungen, Methode und Begründung. Allgemeiner Überblick, praktische Anwendung, *ibid.*, pp. 248-57.
23. ———. Mikrobeobachtungen der Schweisssekretion der Haut des Menschen unter Kontrastfärbung. III, *ibid.*, **149**:157-67, 1925.
24. ———. Mikrobeobachtungen der Schweisssekretion der Haut des Menschen unter Kontrastfärbung. IV. Vergleichende Reizreaktionen unter verschiedener örtlicher Hautdurchblutung, *ibid.*, **155**:342-52, 1927.
25. ———. Mikrobeobachtungen der Schweisssekretion der Haut des Menschen unter Kontrastfärbung. V. Atropinstudien, *ibid.*, **165**:165-79, 1929.
26. KREIDL, A. Die Physiologie der Haut. In: MRACEK, Handb. d. Hautkr., **1**:164-266. Vienna: Alfred Holder, 1902.
27. KUNO, Y. The physiology of human perspiration. London: J. & A. Churchill, Ltd., 1934.
28. KUNO, Y., and IKEUCHI, K. Quoted by KUNO (27).
29. KUNO, Y., and KASHIWABARA, I. An effective method for suppression of local sweating, Chinese J. Physiol., **11**:41-45, 1937.
30. LASZLO, D., and SCHURMEYER, A. Perspiratio insensibilis und Grundumsatz, Ztschr. f. klin. Med., **116**:22-35, 1931.
31. LEVINE, S. Z., and MARPLES, E. Insensible perspiration in infancy and in childhood. III. Basal metabolism and basal insensible perspiration of the normal infant: a statistical study of reliability and of correlation, Am. J. Dis. Child., **40**:269-84, 1930.
32. LEVINE, S. Z., and WILSON, J. R. Respiratory metabolism in infancy and in childhood. IV. Elimination of water through the skin and respiratory passages, Am. J. Dis. Child., **33**:204-12, 1927.
33. LOBITZ, W. C., JR., and OSTERBERG, A. E. Chemistry of palmar sweat; preliminary report; apparatus and technics, J. Invest. Dermat., **6**:63-74, 1945.
34. LOEWY, A., and WECHSELMANN, W. Zur Physiologie und Pathologie des Wasserwechsels und der Wärmeregulation seitens des Hautorgans, Arch. f. path. Anat., **206**:79-121, 1911.
35. MAGENDANTZ, H. Über die Beziehungen zwischen Perspiratio insensibilis und Grundumsatz bei Gesunden und Kranken, Deutsches Arch. f. klin. Med., **173**:44-52, 1932.
36. MELCZER, N. Über die Hautatmung, Dermat. Ztschr., **46**:185-98, 1926.
37. MOOG, O. Der Einfluss der Temperatur auf die unmerkliche Hautwasserabgabe, Ztschr. f. ges. exper. Med., **31**:316-24, 1923.

38. ———. Neuere Untersuchungen über der Perspiratio insensibilis, Verhandl. d. deutsch. Gesellsch. f. inn. Med., **38**:299–301, 1926.
39. ———. Über die Bedeutung der Epidermis für die unmerkliche Hautwasserabgabe, Ztschr. f. d. ges. exper. Med., **54**:226–36, 1927.
40. PALMES, E. D. An apparatus and method for the continuous measurements of evaporative water loss from human subjects, Rev. Scient. Instruments, **19**:711–17, 1948.
41. PINSON, F. A. Evaporation from human skin with sweat glands inactivated, Am. J. Physiol., **137**:492–503, 1942.
42. RANDALL, W. C. Sweat gland activity and changing patterns of sweat secretion on the skin surface, Am. J. Physiol., **147**:391–98, 1946.
43. RICHARDSON, H. B. Clinical calorimetry. XL. The effect of the absence of sweat glands on the elimination of water from the skin and lung, J. Biol. Chem., **67**:397–411, 1926.
44. ROTHMAN, S. Über den Einfluss einiger dermatotherapeutischer Grundsubstanzen auf die insensible Wasserabgabe der Haut, Arch. f. Dermat. u. Syph., **131**:549–67, 1921.
45. ———. Über die Wirkung des ultravioletten Lichts auf die unmerkliche Wasserabgabe der Haut, Strahlentherapie, **35**:381–86, 1930.
46. ———. Zusammenhang der Verhornung mit der insensiblen Perspiration, Zentralbl. f. Haut- u. Geschlechtskr., **37**:30, 1931.
47. ———. Resorption durch die Haut. In: BETHE, Handb. d. norm. u. path. Physiol., **4**:107–51 and **18**:85–91. Berlin J. Springer, 1929 and 1932.
48. ———. The principles of percutaneous absorption, J. Lab. & Clin. Med., **28**:1305–21, 1943.
49. ROTHMAN, S., and SCHAAF, F. Chemie der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1/2**:161–377. Berlin: J. Springer, 1929.
50. SCHWENKENBECHER, A. Die Haut als Exkretionsorgan. In: BETHE, Handb. d. norm. u. path. Physiol., **4**:709–68. Berlin: J. Springer, 1929.
51. SNAPP, R. H. Personal communication.
52. SODEMAN, W. A., and BURCH, G. E. Regional variations in water loss from skin of diseased subjects living in subtropical climate, J. Clin. Investigation, **23**:37–43, 1944.
53. SODERSTROM, G. F., and DU BOIS, E. F. Clinical calorimetry. XXV. The water elimination through skin and respiratory passages in health and disease, Arch. Int. Med., **19**:931–57, 1917.
54. SUNDERMAN, F. W. Persons lacking sweat glands; hereditary ectodermal dysplasia of the anhydrotic type, Arch. Int. Med., **67**:846–54, 1941.
55. TROLLE, C. Study of the insensible perspiration from the skin of the dog, Skandinav. Arch. f. Physiol., pp. 225–46, 1937.
56. UNNA, P. G. Kritisches und Historisches über die Lehre von der Schweisssekretion. In: SCHMIDT, Jahrb. d. inn.- u. ausländischen ges. Med., **194**:89–99, 1882.
57. UPSHAW, B. Y., and MONTGOMERY, H. Hereditary anhidrotic ectodermal dysplasia; clinical and pathologic study, Arch. Dermat. & Syph., **60**:1170–83, 1949.
58. VASTI, A. The insensible water loss through the skin, Am. J. Physiol., **102**:60–70, 1932.
59. WHITEHOUSE, A. G. R.; HANCOCK, W.; and HALDANE, J. S. Osmotic passage of water and gases through human skin, Proc. Roy. Soc. London, s.B, **111**:412–29, 1932.
60. WHITEHOUSE, A. G. R., and RAMAGE, H. The permeability of human skin to electrolytes, Proc. Roy. Soc. London, s.B, **113**:42–48, 1933.
61. WILEY, F. H., and NEWBURGH, L. H. Relationship between environment and basal insensible loss of weight, J. Clin. Investigation, **10**:689–701, 1931.
62. WINSOR, T., and BURCH, G. E. Differential roles of human epigastric skin on diffusion rate of water, Arch. Int. Med., **74**:428–36, 1944.

CHAPTER 10

The Role of the Skin in Thermoregulation¹

I. GENERAL	244
II. HEAT LOSS THROUGH THE SKIN	245
A. Radiation	245
B. Convection	247
C. Conduction	247
D. Evaporation	248
III. ROLE OF HUMIDITY AND THE RELATIVE HUMIDITY OF THE SKIN	248
IV. NONTHERMOREGULATORY EVAPORATIVE HEAT LOSS	251
V. THERMAL GRADIENTS	251
VI. RELATION OF INTERNAL AND EXTERNAL GRADIENTS	253
VII. VASOMOTOR CHANGES SERVING THERMOREGULATION	253
VIII. FACTORS INFLUENCING SKIN SURFACE TEMPERATURE	258
A. General	258
B. Influence of Surface Pattern	258
C. Influence of Sebum	258
D. Regional Differences	258
E. Influence of Clothing	259
IX. HEAT REGULATION IN DISEASED SKIN	261
X. LOCAL EFFECTS OF COLD	261
XI. PENETRATION OF THERMAL STIMULI	264
XII. CHANGES IN SWEAT SECRETION DURING ACCLIMATIZATION TO HIGH ENVIRONMENTAL TEMPERATURES	265

I. GENERAL

HEAT is continuously produced in the body by metabolic processes, oxidation of carbohydrates and fats being the chief source of energy. In homeothermal animals and man the loss of heat is continuously adjusted to heat production in order to main-

tain heat balance and constant body temperature. The center of this involved regulatory mechanism resides in the hypothalamus. The old theory of Meyer (56) has remained adequate in most respects (5). This theory postulated the existence of two opposing centers, one in the anterior hypothalamus, controlling heat dissipation, and one lying more posteriorly, controlling heat con-

1. In this chapter the author has freely used the material presented in the monographs of Thauer (83), Sheard (73), Du Bois (24), and Newburgh (61).

servation. Presumably, the former center is stimulated by sensations of warmth (p. 100) and has an optimal temperature for activity which lies above the normal hypothalamic temperature, while the latter is stimulated by sensations of cold and has an optimal temperature below the normal level. Reactions of these centers are reinforced and maintained also by local reactions of cutaneous and deeper blood vessels. Under basal conditions, at absolute rest and in fasting, a thermal balance can be maintained at which heat production and heat loss are equal without further adjustment. When the environmental temperature is between 25° and 31° C. (77°–88° F.), a clothed semireclining subject will easily maintain heat balance by cutaneous vasomotor adjustment (zone of vasomotor control). Above this range, at an air temperature of between 31° and 32° C. (88°–90° F.), when cutaneous blood flow has reached its maximum, sweating sets in (zone of evaporative regulation). Between 31° and 36° C. (88°–98° F.) and at low humidity, evaporative heat loss easily maintains normal body temperature. Below the zone of vasomotor control the skin temperature falls and body temperature is maintained chiefly by chills (zone of cooling).

The thermostatic regulation of the human body is certainly not perfect. When the atmospheric temperature increases suddenly, there is a lag in vasomotor adjustment and later a lag in the onset of sweating, so

that a temporary hyperthermia results. But even under standardized atmospheric conditions there is no "normal" body temperature, only a normal range (46). At the same time, different temperatures are present in different parts of the body, and, in addition, the skin and a large mass of the subcutaneous fat tissue averages 2°–4° C. cooler than the interior.²

The well-known diurnal variations of body temperature are not well understood but probably are associated with the cycles of sleep and wakefulness and with corresponding variations in metabolism. It has not been investigated, but it is probable that exacerbations of itching in inflammatory skin diseases at certain hours of the day, usually in the late afternoon, have something to do with peak body temperatures, and associated increased skin temperatures with increased cutaneous blood flow in that period of the day.

In nonbasal states, adjustments are needed to counteract increased heat production. Both exercise and food intake increase heat production, exercise by directly increasing metabolism and food intake by its specific dynamic action. Intensified itching and intensified objective signs in inflammatory conditions of the skin after food intake may be due to increased cutaneous blood flow and increased skin temperatures, which follow increased heat production.

II. HEAT LOSS THROUGH THE SKIN

Heat loss through the skin surface takes place by four different physical mechanisms: radiation, convection, conduction, and evaporation.

A. RADIATION

Radiation represents transfer of energy from the surface of the skin to the environment by electromagnetic waves, which travel in a straight line with the speed of light. It is expressed as total radiant energy per second per square centimeter. Like any object that is warmer than its surroundings, the skin radiates heat to the environment in

the form of infrared rays. The spectrum of infrared radiation of the human skin extends from about 5 to 20 μ , with a maximum at about 9 μ (36) (Fig. 1). The radiation from human skin corresponds with the radiation of a thermal "black body" at 300° K.³ In the

2. For different temperature levels within the body see Bazett *et al.* (7) and Eichna *et al.* (27).

3. K. = Kelvin scale for absolute temperature. The absolute zero point is -273° C.; 300° K. corresponds with 27° C. A thermal black-body radiator absorbs all incident radiation and emits a radiation which is proportional to the fourth power of its absolute temperature.

region of the emitted infrared rays there is no difference between the radiation from the skin of a white man and that from a very dark man of the Negro race. Both are approximately 99 per cent perfect black bodies

The amount of energy lost by radiation from the skin amounts to about 60 per cent of the total heat loss under ordinary atmospheric conditions. Its magnitude depends upon the difference between the tempera-

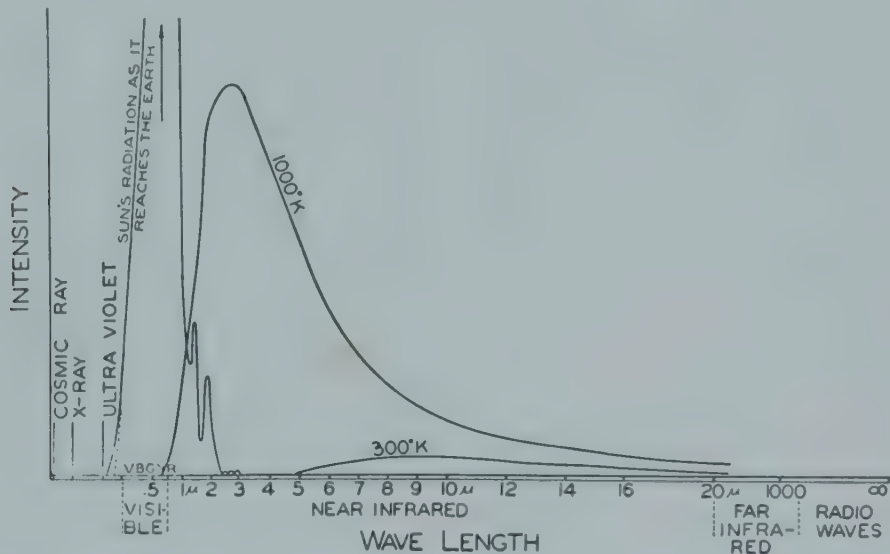


FIG. 1.—Spectral distribution of the thermal radiation from the sun ($6,000^{\circ}\text{K.}$), a red-hot stove ($1,000^{\circ}\text{K.}$), and the human body (300°K.). Modified from Hardy and Muschenheim. From Newburgh (61). (Reproduced by permission of W. B. Saunders Company.)

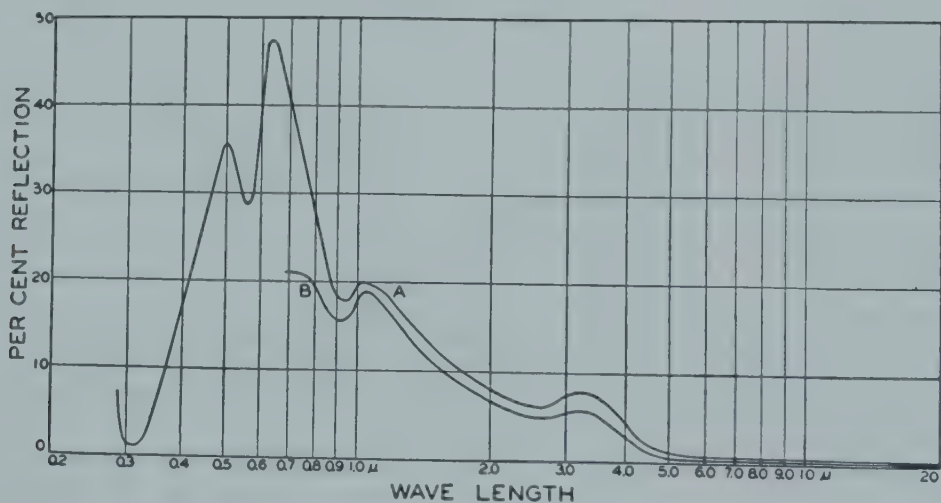


FIG. 2.—Reflecting power of white and Negro skin. A, white skin; B, Negro skin. Modified from Buttner and Hardy and Muschenheim. From Newburgh (61). (Reproduced by permission of W. B. Saunders Company.)

in the infrared region (36) (Fig. 2). Only 1 per cent of incident infrared radiation is reflected from white and from dark skin, and the differences within this 1 per cent reflection are negligible. Visible light reflection, of course, is greater in the white race.

ture of the skin surface and that of the environment and on the size and nature of the radiating surface. Whereas measurements have been made on radiation of normal skin in all regions (13), little is known about the changes in radiation associated with skin

diseases in which the surface relief is modified, as is the case in a great variety of hyperkeratotic and scaling lesions, in lichenified areas, etc.

B. CONVECTION

Convection results from transfer of energy by means of moving air. Under ordinary circumstances, air is cooler than the skin surface. If there is no air movement, the air layer in immediate contact with the skin is warmer than the air of the atmosphere. This air layer, the thickness of which is not known, is often referred to as the "private climate." If there is a draft, the warm air near the skin is continuously replaced by cooler air, and thus heat is carried away from the skin surface to the environment. As Sheard (73) points out, a "semiprivate climate" can be created by clothing, although most clothes are fairly open to air currents and moisture (p. 259). It is known that under ordinary circumstances not only the temperature but also the humidity of the "private climate" are higher than those of the atmosphere. This is the "vapor mantle" (or *Dunsthülle*) of Unna (84).

Cooling of the skin by air movement is most pronounced at low atmospheric temperatures. At 26° C. (78° 8 F.) an air current of 0.5 meter/sec directed at the face lowers its skin temperature by 2° C. (53). Convection causes cooling not only by carrying warm air away but also by increasing evaporation of sweat.

If the air is warmer than the skin surface, heat is not lost but is gained by convection. But this gain might be upset by heat loss due to increased evaporation. At 34° C. (93° F.) atmospheric temperature, the higher the air velocity, the easier the body can adjust itself to high relative humidity. But at a temperature of 41° C. (106° F.) the situation is reversed: apparently the heat gained by air velocity is too great to be counterbalanced by increased evaporation (73). Du Bois (23) found that in the case of a nude subject in the basal state in a calorimeter at 22° 7 C. (73° F.) 66 per cent of the total heat loss was accounted for by radiation, 19 per

cent by evaporation, and only 15 per cent by convection. Clothing did not change these percentages appreciably. However, when a fan was used in the calorimeter at 29° C., the relative percentage of heat lost by radiation decreased to 41 per cent, that lost by convection increased to 33 per cent, and evaporation accounted for 26 per cent. Decrease of radiation in this case was due to lowering of the skin surface temperature by convectional heat loss.

The role of chronic exposure to wind in the development of pathologic cutaneous manifestations has often been suspected, as the expression "weatherbeaten skin" indicates; but this effect has never been experimentally investigated. In the development of "sailor's skin" or "farmer's skin," wind, in addition to the exposure to ultraviolet rays, may or may not play a role. Cooling by wind is associated with vasoconstriction of the minute vessels of the skin; and it may well be that the epidermal and connective-tissue changes in "weatherbeaten skin" are enhanced by nutritional disturbances caused by unduly frequent and long-lasting vasoconstriction if at the same time the skin is exposed to ultraviolet radiation.

C. CONDUCTION

Conduction is transfer of heat from one medium to another when the two media are in direct contact with each other. As contrasted with convection, no movement of either medium is involved in conduction. In physiological experimentation heat loss by conduction plays a relatively minor role (24), because conventional clothing does not essentially change physical heat loss (p. 259). From the skin-hygiene point of view, conduction of heat from skin to heat-insulating material, such as rubber sheets, is of great significance. The insulating material is rapidly warmed up to skin temperature by conduction and hinders further heat loss by radiation, convection, and evaporation. This is often the situation in hospital beds equipped with rubber sheets under or above the bed sheet. Similar but less conspicuous heat stasis may occur even without rubber

sheets when the bedding material does not conduct heat well.

D. EVAPORATION

Evaporation of water from the skin surface causes heat loss because transformation of water into vapor requires heat that is taken away from the skin surface. At an environmental temperature of about 31° C. there is a general sweat outbreak. This "critical atmospheric temperature" is somewhat different in different individuals; in the same individual it drops several degrees in the process of acclimatization to heat (51) (p. 265). The critical skin temperature at which sweating is initiated in the resting subject is given as being between 31° C. (73) and 34.5° C. (86, 6).

It has been known that sweating elicited by exercise starts at a lower skin temperature than sweating produced by external heat. Bazett (6) dealt with this phenomenon in great detail. He found that the first line of defense against atmospheric heat is vascular readjustment, while thermoregulation is

maintained during exercise primarily by sweating. He developed the theory that, in addition to the superficial thermal receptors lying adjacent to the superficial vascular plexuses (Ruffini and Krause endings, p. 123), there are deep-seated heat receptors at the dermal-subdermal junction. These receptors, he postulated, are situated adjacent to the deep vascular plexus. The superficial receptors and their adjacent vessels are affected mainly by external heat, and the deeper ones by internal heat from working muscles. Superficial heat receptors are concerned with vasomotor adjustments, and the deep ones with sweating. The latter are more readily stimulated by internally produced heat. The adequate stimuli for both types of heat receptors are thermal gradients (p. 146), and the relation of sweating to skin temperature depends on these gradients and their topography.

In exercise there is a stricter control of body temperature than in a hot atmosphere, but in exercise the homeostasis of the body temperature is at a higher level (6).

III. ROLE OF HUMIDITY AND THE RELATIVE HUMIDITY OF THE SKIN

Sweating is useful for thermoregulation only as long as the sweat can evaporate. In a dry, hot environment, evaporative cooling is a most efficient regulatory mechanism, particularly in acclimatized persons whose sweat-production capacity is greatly increased. Increase in evaporative cooling in these individuals accounts for 75–90 per cent of the reduction in body heat, whereas a decrease in metabolic heat production ("chemical heat regulation") accounts for only 5–20 per cent (28). However, in humid heat, sweating is grossly inefficient because up to two-thirds of the sweat may run off the skin surface without being evaporated. Water and salt are wasted without any benefit. Profuse sweat secretion continues, and severe dehydration and salt deprivation may set in, whether the sweat can evaporate or not (26).⁴

The evaporative cooling mechanism has

two more dissimilar features (28): First, it lowers the skin temperature and thereby increases heat gains by convection and radiation from the outside; twice as much sweat is required to counteract this effect as would be necessary if the skin temperature had not fallen. Second, a fall in skin temperature widens the internal thermal gradient (p. 251), and therefore a smaller volume of peripheral blood flow is used for transferring deep heat to the periphery; the blood flow is 30 per cent less than it would have been without a drop in skin temperature (28). The

4. Only at an extremely high rate of sweating (1,400 gm/hr or more) in severe heat and under strenuous exercise is there a decline in sweat secretion after the second hour. This decline is greater in humid than in dry heat. Administration of water or salt and keeping the skin temperature constant do not prevent this decline. Therefore, it must be interpreted as a fatigue of the sweating mechanism in some way (p. 213) (34, 66).

evaporative heat loss at low environmental temperatures is insignificant percentage-wise. At air temperatures up to 24° C. (75° F.) it makes little difference for a resting person whether the atmosphere is humid or dry (25). Du Bois and Hardy give the following explanation for this phenomenon:

Assuming constant air motion, the rate of water evaporation depends upon the difference in vapor pressure of water on the skin and in the air. The vapor pressure on the skin is determined by, and can be calculated from, the skin temperature. The vapor pressure of the atmospheric water is determined by the air temperature and relative humidity. Thus the evaporating tendency of water on the skin can be computed for any humidity. For low atmospheric temperatures it is found that, in spite of high relative humidity, full evaporative loss can take place. For instance, at 21° C. (70° F.) room temperature and 80 per cent relative humidity, the air vapor pressure is only 0.58 inches Hg, whereas on the skin, with a skin surface temperature of 33° C. (91° F.), the pressure is 1.47 inches Hg. This gradient permits full evaporative loss. Of course, the rate of evaporation is somewhat slower at high humidity even at low atmospheric temperatures, but the difference is slight. At 0° C. (32° F.) a change from 50 to 100 per cent humidity affects the evaporative rate by only 6 per cent, and at 14.5° C. (60° F.) by 20 per cent. At 35° C. (95° F.), however, such a change in humidity will cause complete cessation of sweat evaporation.

For a resting person, air humidity begins to be of importance between 30° and 31.4° C. (86° and 88° F.) air temperature, at which point the difference between 50 and 100 per cent relative humidity decides whether the person will be comfortable or will develop hyperthermia. For a moderately active person, one who is walking slowly, for instance, the importance of humidity rises rapidly from 25° C. (78° F.) on and may cause serious trouble at about 32° C. (90° F.). With increasing activity, humidity becomes an important factor in an increasingly lower temperature range. If a person runs fast at 22°

C. (72° F.) and 100 per cent relative humidity, he may suffer a heat stroke.

The reduction in evaporation of sweat at high humidity is of great importance not only for thermoregulation but also for the health of the skin. In humid heat the non-evaporating sweat on the skin causes occlusion of sweat pores, with all the consequences of the sweat-retention syndrome (pp. 270 ff.). Furthermore, at equally high air temperatures, the arterial blood flow to

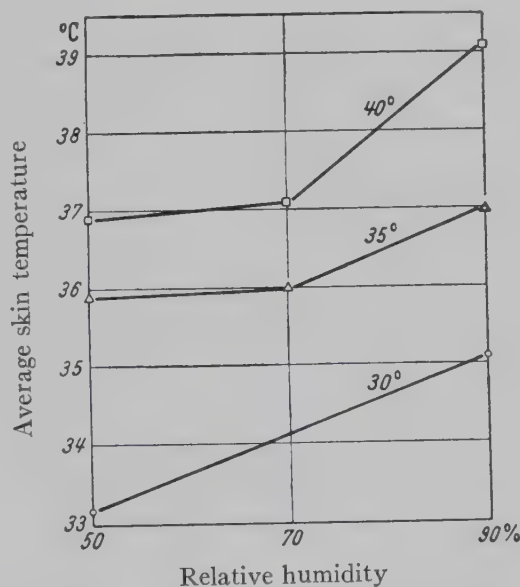


FIG. 3.—Curves showing average skin temperature as a function of relative humidity after 2½ hours in environmental temperatures of 30°, 35°, and 40° C. From Neuroth (60). (Reproduced by permission of Springer-Verlag.)

the skin and the skin surface temperature are much higher in a humid than in a dry atmosphere, because the cooling effect of sweat evaporation is lacking at high humidities. This is shown in Figure 3 taken from Neuroth's (60) experiments. The adverse effect of prolonged arterial hyperemia of the skin in the case of inflammatory skin diseases is obvious.

Winslow, Herrington, and Gagge (86) have dealt with a special phase of evaporative heat loss which is particularly important for the physiology of the skin. Whereas at low air temperatures the relative insignificance of air humidity can be explained physically (see above), at somewhat higher

temperatures, but still below the critical temperature of visible sweat secretion, such an explanation is unsatisfactory. At 30° C. (86° F.) the evaporative heat loss is still at an almost minimum level of 15–25 kg-cal/hr, corresponding with the water evaporation from nonsweating normal skin. At this temperature, conditions can be created at which vapor pressure in the air becomes equal with the vapor pressure of water on the skin; at this point evaporation should come to a standstill. However, evaporative heat loss does not decrease with increasing humidity, and body temperature remains constant. Some compensatory physiological mechanism has to be assumed to account for the constant rate of evaporation in spite of the decreasing “physical” rate. Winslow *et al.* (86) assumed that the compensation is achieved by an increasing *wettedness of the skin*, i.e., by an increase in the percentage of the skin surface which is wetted.⁵ It was concluded that the degree of wettedness changes so as to maintain the same evaporative heat loss at all humidities. Accordingly, from 24° to 30° C. the wettedness percentage increases at high humidity but not at low humidity.

Mole (57), reviewing this work, found that the wettedness as defined by Gagge does not vary consistently with the humidity of the air. Therefore, he substituted the concept of *relative humidity of the skin* (rh_s), which is defined as the ratio of vapor pressure on the skin surface ($v.p._s$) to vapor pressure of air when saturated at skin temperature ($e[T_s]$), or, with mathematical symbols,

$$\frac{v.p._s}{e(T_s)} = rh_s.$$

This rh_s may be taken as a measure of the water content of the surface layer of the skin, expressed as a percentage of the maximum when the skin surface is covered by a continuous film of water. It is thus

5. The concept and mathematical formulation of “wettedness” was developed by Gagge (31).

analogous to the concept of relative humidity of the air. It measures the partial pressure of water vapor at the skin surface as a fraction of the maximum saturation value. Of course, the skin surface is not so homogeneous as air, and therefore rh_s can be only an average figure. Also, rh_s is different on different parts of the body.

Mole (57) finds that rh_s varies as it should if transepidermal water loss is a simple diffusion process which depends on the removal of water vapor by the environment. At constant air temperature and convection, an increase in air humidity will temporarily decrease the loss of water from the skin surface. But the continuing upward diffusion of water within the skin will raise the skin humidity and hence the evaporative loss of water to the point where this once more equals the rate of water transport by diffusion through the skin. This regulation is facilitated by temperature changes in the skin. When, because of high air humidity, evaporation is decreased, the skin temperature rises, the thermal gradient between skin surface and air increases, and evaporation is thereby facilitated.

Increased metabolism with increased heat production puts into operation regulating mechanisms similar to those caused by temperature changes in the surrounding air. The warm and moist skin in hyperthyroidism and the dry and cool skin in myxedema are primarily the results of modified thermoregulating vasomotor and secretory reactions due to changes in metabolic heat production. However, it seems that in hyperthyroidism there is, in addition to increased metabolism, a primary augmented responsiveness of sweat glands (p. 165).

In peripheral vascular diseases, when vasomotor heat regulation is disturbed because of deficient vasodilator capacity of vessels on the distal parts of the extremities, cold and moist hands and feet are rather characteristic features. Sheard (73) assumed that sweat secretion in these conditions occurs in order to compensate for the inability to dissipate heat by vasodilatation.

IV. NONTHERMOREGULATORY EVAPORATIVE HEAT LOSS

From the dermatological point of view it should be emphasized that visible sweat secretion and heat loss by sweat evaporation often occur also in the comfortable zone of "vasomotor control" and even in the "zone of cooling," quite independent of thermoregulatory needs. Sweating can be elicited

with great ease at air temperatures below 31°C .—for instance, by administration of hot drinks (probably an example of viscerocutaneous reflex) and, of course, by a great variety of nonthermoregulatory nervous impulses, the most important phenomenon being emotional sweating.

V. THERMAL GRADIENTS (8)

Under normal circumstances the internal temperature is higher than that of the environment, and there is a thermal gradient from the inside of the body to the surrounding air. The gradient has two parts (15): (1) from the interior to the skin surface (internal or physiological gradient) and (2) from there (through clothing) to the surrounding air (external or physical gradient).

Under comfortable atmospheric conditions the average skin surface temperature is about 5°C . lower than that of the inside of the body. Heat flows steadily from the inside peripherally by means of heat conduction, the flow depending on the specific thermal conductivity of the tissues. This conductivity is great in all internal organs, and the temperature at 2.5 cm. below the surface is still about the same as anywhere in the interior of the body, because heat flows freely in all directions from the sites of heat production. The site of the actual physical gradient of 3°C . or more in a cool environment is the subcutaneous fat tissue and the skin (8) (Fig. 4). The average gradient in this zone is $2^{\circ}\text{C}/\text{cm}$, which corresponds with a much lower conductivity than that of water or glass, for instance. It is even poorer than that of rubber. In other words, physically the subdermis and the skin are efficient insulators which hinder the equalization of inside and outside temperatures.

The fall of temperature within subdermis and skin is not continuous. Discontinuity results from the different thermal conductance of the different layers. The poorest conductors are the horny layer and the subcutane-

ous fat tissue. Both layers may maintain considerable difference in temperature between their upper and lower limits. Over caluses the skin surface temperature is low,

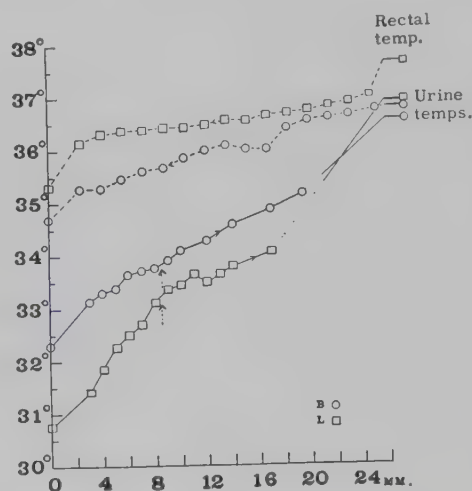


FIG. 4.—Thermal gradients in the forearm in two subjects on two occasions; ordinates indicate temperature in $^{\circ}\text{C}$., and abscissae the depth in the skin of the measuring thermocouple in millimeters. The two upper curves represent subjects *B* and *L* on a hot summer day (dry-bulb temperature, 29.1°C .; effective temperature, 23.9°C .). The two lower curves represent similar gradients determined on a cool autumn day (dry-bulb temperature, 19°C .; effective temperature, 17.6°C .). Rectal and urine temperatures are indicated in the upper right corner of the graph. Arrows indicate the observed depth of the fascia. From Bazett and McGlone (8). (Reproduced by permission of the American Physiological Society and Mrs. Bazett.)

because the hyperkeratotic horny layer hinders the outward flow of the internal heat. Similarly, a thick subcutaneous fat layer hinders dissipation of heat. The conductance of obese persons' subdermis plus skin is

about one-third lower than that of slender persons—hence the relative coolness of the buttocks and higher average skin surface temperatures in slender, than in obese, persons. The thermal conductance of the skin may also vary according to variations in water and blood content. Water conducts heat twenty times better than the dry horny layer does, and therefore imbibition of water by the horny layer increases heat conductance considerably. A physiological regulation mechanism may be seen in the fact that sweat moistens the horny layer, thus enhancing the cooling effect of evaporation by increased heat conductivity.

The perception of warmth and cold depends on the heat conductance of the skin. Thermal sensory thresholds are lowered when the horny layer is moist, because the thermal stimulus penetrates more easily from the outside to the deep-seated receptor organs through moist than through dry horny layer. Indirectly, all other sensory perceptions may also be influenced by moisture of the horny layer. When the outside temperature is high and heat penetrates with ease through a moist horny layer, reactive hyperemia develops more readily, and hyperemia, in turn, possibly causes lowering of all thresholds of sensory perceptions. Hyperemic skin conducts heat better than anemic skin. Still, even with maximal blood flow, the skin remains an effective insulator.

Hardy and Soderstrom (37) differentiate two forms of internal heat conductivity, one being physical, the other physiological. Between 23° and 28° C. the conductivity is purely physical, i.e., it is determined by the physical (structural) properties of the tissues without interference of physiological reactions. Above 28° C., with the onset of active vasodilatation, the peripheral blood flow increases almost proportionately to the environmental temperature, until at 35° C. skin surface temperature it reaches 0.23 l/min, or 10 per cent of the basal cardiac output. This physiological reaction increases heat conductance considerably.

In their investigations of thermal injury Henriques and Moritz (38, 58) measured

separately the heat conductivity of heat-separated epidermis, corium, subcutaneous fat, and subcutaneous muscle of pigs. Expressing thermal conductivity, K , as

$$\frac{\text{calories} \times \text{cm.}}{\text{cm.}^2 \times \text{min.} \times ^\circ\text{C.}},$$

they obtained the following average values: epidermis, 0.03; dermis, 0.053; subcutaneous fat, 0.023; and muscle, 0.066.

Even if the skin surface is suddenly brought to and maintained at temperatures from 45° to 100° C. by contact heat, it takes a measurable time (0.5–1 minute) to warm the epidermis through to the basal layer, because of its relatively poor conductivity. After about 1 minute the epidermis is heat-saturated, i.e., from then on, the amount of heat entering and leaving the epidermis will be balanced. In this steady state Henriques and Moritz computed the temperature of the epidermal-dermal interface at different surface temperatures, as shown in the accompanying tabulation.

Surface	45° C.	55° C.	65° C.
Interface	44.8 C.	54.5 C.	64.2 C.

If heating is achieved not by immediately raising the surface temperature but by surrounding the entire animal with an envelope of ambient air and radiant heat between 80° and 175° C., the warming of the epidermis is extremely slow. Under these conditions, even after a heat exposure of 15 minutes, the interface temperature is far lower than the temperature of the heat source, and the animal succumbs to hyperthermia long before the temperature of the epidermis approaches that of the air.

A heat source which raises the surface temperature suddenly to that of the heat source itself has, on a time basis, at least a thousand times greater propensity to injure the epidermis than has a heat source which raises the skin temperature by means of radiation and conduction and convection of relatively immobile air.

VI. RELATION OF INTERNAL AND EXTERNAL GRADIENTS

Burton (16) introduced the term "thermal circulation index" for the ratio of external to internal gradient (p. 251). The body temperature being constant, this ratio is determined by the skin surface temperature and the external gradient. Both these values depend primarily on the degree of peripheral blood flow. Hence the term "thermal circulation index." In addition, the index depends on the thickness of the different layers of skin and subdermis. Therefore, the index is different in different areas, even after air temperature, humidity, ventilation, and clothing have been standardized (Table 1). The table shows that skin temperatures as well as indices are higher on trunk and forehead than on extremities. It is also seen that temperature and index decrease on the lower extremity in the distal direction.⁶

Elevation of the index means increase in heat conductivity either by increased blood flow or by another type of hydration of the skin. Physiologically, the variability of the index is of the order of only 20 per cent for the trunk and 30 per cent for the leg in a wide range of environmental temperature. However, in erythematous skin the index may be increased four- to fivefold. Because of increased blood flow, the skin surface temperature is substantially elevated and has been brought closer to body temperature; thus the ratio of the external to the internal gradient has also greatly increased. On urticarial wheals the index is increased twofold because of hydration and subsequent increased conductivity.

A great increase in the index was found in general anesthesia (15), in which there is maximal blood flow to the skin (45) which

cannot be further increased by sympathectomy (74). In spite of the maximal blood flow, the skin color remains normal, because the blood passes through the superficial capillaries rapidly without dilating them or flows in large amounts through preferential

TABLE 1

SKIN TEMPERATURES AND THERMAL CIRCULATION INDICES ON SUBJECT AFTER LYING NUDE FOR 20 MINUTES AT ROOM TEMPERATURE OF 22°8 C.

(Rectal Temperature 37°25 C.) (15)

Body Region	Skin Temp. (° C.)	Drop from Skin to Air (° C.)	Drop from Interior to Skin (° C.)	Circulation Index
Forehead.....	33.40	10.60	3.85	2.75
Clavicle.....	33.60	10.80	3.65	2.96
Over breast.....	32.75	9.95	4.50	2.21
1 in. over umbilicus	34.20	11.40	3.05	3.75
Over apex of heart.	33.30	10.50	3.95	2.67
Lumbar region....	33.30	10.50	3.95	2.67
Arm, biceps.....	32.85	10.05	4.40	2.28
Palm of hand.....	32.85	10.05	4.40	2.28
Kneecap.....	32.35	9.55	4.90	1.95
Calf of leg.....	32.20	9.40	5.05	1.86
Sole of foot.....	30.20	7.40	7.05	1.05
Big toe.....	30.95	8.15	6.30	1.29

channels only (p. 63). In general anesthesia the index on the toes may increase by a factor of 12 (15).

Hensel (40) found at room temperatures of 20°–22° C. a gradient of 0°2–0°5 C. per millimeter in the most superficial layers of the skin. By suddenly raising or lowering surface temperature, he determined a heat-conduction ratio for different layers.

VII. VASOMOTOR CHANGES SERVING THERMOREGULATION

If heat production is increased or the atmospheric temperature is raised in the zone of vasomotor control, there is a shift of blood flow from the interior to the tegument. Conversely, if metabolism is lowered or the atmospheric temperature is decreased, pe-

ripheral vasoconstriction ensues, and blood is shifted from superficial to deeper tissues. This is achieved by changes of the vascular

6. Decrease of skin temperature from hip to toe was previously reported also by Foged (30) and by Craig, Horton, and Sheard (21).

tone by reflex mechanisms (p. 87). Although both the vasodilator and the vasoconstrictor reflexes affect the peripheral vessels all over the body surface, the distal parts of the extremities play the most outstanding role in this regulation.

In the zone of cooling, below 25° C. (77° F.) air temperature, the temperature of the skin of toes and fingers is lower than anywhere else on the body surface. At 25°–26° C. room temperature, the skin temperature of fingers approaches that of the forehead and thorax, e.g., 32°–35° C. (89.5°–95° F.), whereas the toes still remain colder by 7°–

It does not make any difference whether increased heat loss from the skin is required on account of high atmospheric temperatures or on account of greater heat production. If the metabolism is increased by the specific dynamic action of food, the same play of vasomotor regulations can be observed (Fig. 7).

In spite of the spectacular role of the "acrae" in these regulations, it is obvious that the vasomotor reactions also occur in other parts. Sheard (73) clearly showed that vasodilatation and consecutive temperature increase take place also on nonacral parts,

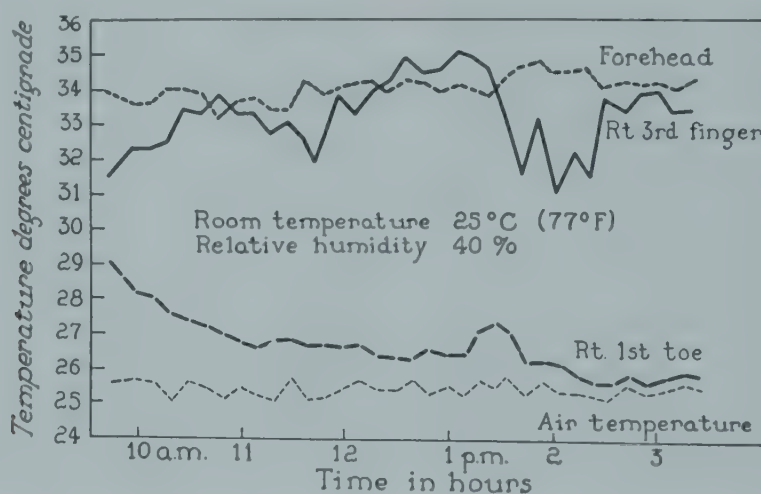


FIG. 5.—Curves of the skin temperatures of the forehead, finger, and toe of a normal subject in the basal metabolic state in an environmental temperature of 25° C. (77° F.) and 40 per cent humidity. From Sheard, Williams, and Horton (75). (Reproduced by permission of Reinhold Publishing Corporation.)

10° C. In this zone the distal parts of the lower extremities act to conserve the heat of the body; finer regulations are controlled by fingers and hands. Above 26° C. (79° F.) room temperature, the blood vessels of the toes also dilate; and at 29°–32° C. (84°–89.5° F.) room temperature, the skin temperature of the toes closely approximates the skin temperature on forehead, thorax, legs, and arms. All this is excellently illustrated in Figures 5 and 6, taken from experiments by Sheard *et al.* (75).

According to responsiveness to cooling, Neuroth (60) distinguishes three types of regions: (1) skin temperatures of forehead, chest, and epigastrium change least; (2) arms and legs take an intermediate position; and (3) hands and feet are cooled most.

such as thighs and forehead. The temperature increase amounts to 2°–4° C. when the room temperature is raised from 20° to 34° C. (68°–93° F.). This increase of 2°–4° C. may make quite a difference in diseased skin by aggravating inflammatory conditions and increasing sensory perceptions.

Horton, Sheard, and Roth (44) studied the vasomotor reactions to alcohol intake. Here vasodilatation occurs first in the face rather promptly, then in the upper extremities, and only late, if at all, in the lower extremities (Fig. 8). Generalized vasodilatation without visible hyperemia is well known to occur after alcohol intake. In drunken persons vasomotor adjustments are paralyzed, and therefore they succumb easily when exposed to severely cold temperatures. The

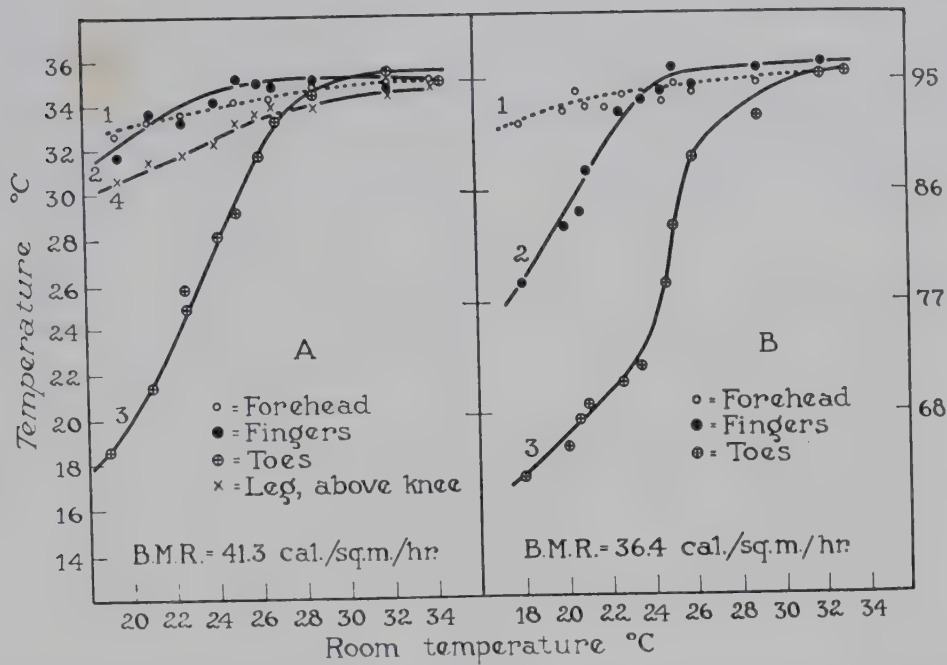


FIG. 6.—Curves showing the relative roles of the upper and lower extremities in the dissipation of heat, as indicated by skin temperatures of fingers and toes of two normal subjects (*A* and *B*) in environmental temperatures from 18° to 35° C. From Sheard, Williams, and Horton (75). (Reproduced by permission of Reinhold Publishing Corporation.)

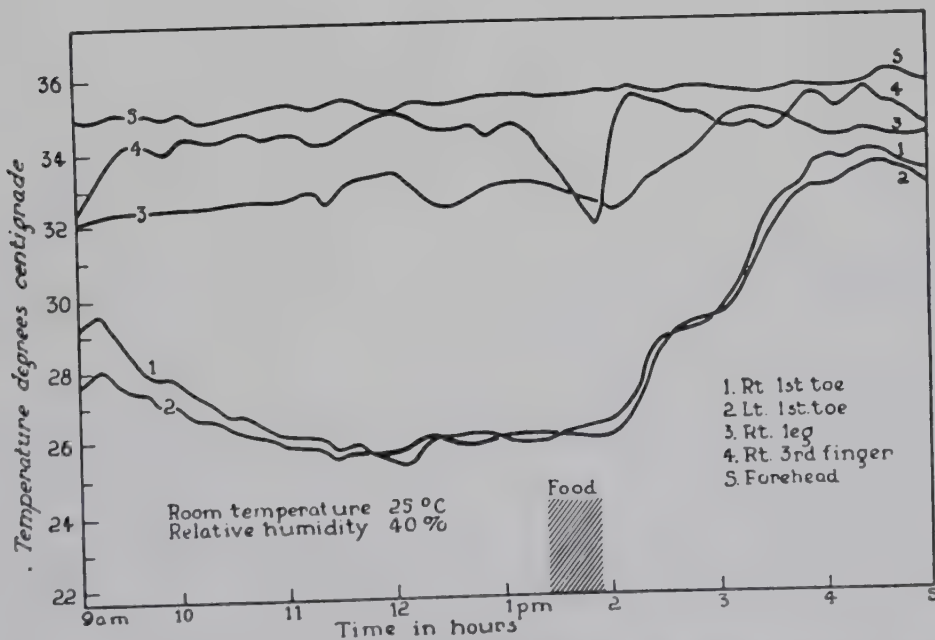


FIG. 7.—Curves showing the time-temperature relationship of the forehead, upper leg, fingers, and toes of a normal subject before and after the ingestion of food. Curves 1 and 2, indicating the temperatures of the toes, show the role of the lower extremities in the control of the dissipation of heat when the subject is in an atmospheric temperature of 25° C. (77° F.) before and after an increase in heat production. From Sheard, Williams, and Horton (75). (Reproduced by permission of Reinhold Publishing Corporation.)

suddenness of increased blood flow to the face suggests that this is a viscerocutaneous reflex rather than a chemical-pharmacological effect of the alcohol circulating in the blood stream. The rationale of alcohol abstinence in rosacea and other diseases with pathologically decreased vasomotor tone is evident.

In the zone of cooling, under basal conditions with the person lying naked and motionless, the greatest vasomotor change

temperature of the skin and of the surrounding air. Loss of heat per unit time is proportional to the surface area.

The mechanism whereby these vascular reactions and changes in blood distribution maintain body temperature is this: At high atmospheric temperatures or with elevated heat production, increased blood flow to the skin effects more rapid transport of heat to the surface; at the same time, the heat conductivity of the skin increases; the skin sur-

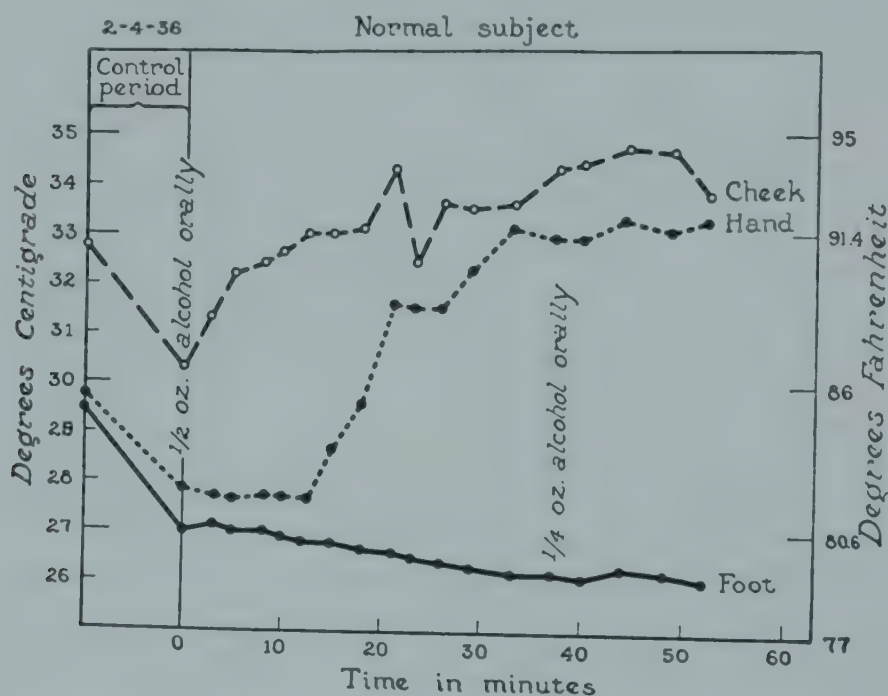


FIG. 8.—Surface temperatures of cheek, hand, and foot, illustrating a vasomotor gradient, following the oral administration of $\frac{1}{2}$ ounce (15 cc.) of alcohol. Vasodilatation occurred promptly in the cheek but was somewhat delayed in the hand; no vasodilatation occurred in the foot. From Horton, Sheard, and Roth (44). (Reproduced by permission of Dr. Horton and the Medical Society of New Jersey.)

again takes place in the feet and toes (Fig. 9). Figure 9 shows that there is a rapid drop of skin temperature on the feet by as much as 8°C ., whereas the average skin temperature drops more gradually and, in all, not more than 4.5°C . Roughly, cooling of the room by 1°C . causes a drop of $\frac{1}{2}^{\circ}\text{C}$. in the average skin temperature (35). In spite of vasoconstriction, the insulating power of the skin does not change essentially in this zone. The fall in average skin temperature seems to be the effect of simple physical cooling (73), according to Newton's law: the rate of cooling is proportional to the difference in

face temperature becomes elevated, and heat loss by convection and radiation increases. Also, blood is being cooled more efficiently because larger volumes get into the skin. Decreased peripheral blood flow acts in the opposite direction. In the protection against cold the capillaries contract and, particularly in the acrae, the arterial blood returns through arteriovenous anastomoses (p. 77) into deep veins, so that, in spite of lowered skin temperature, not much of the heat is lost by cooling of the blood.

In thermoregulatory vasomotor reactions it is the total volume of blood per unit time

flowing through the entire thickness of the skin which counts. Whether or not the superficial minute vessels dilate is not important (p. 69). It is rather characteristic that maximal peripheral blood flow is frequently found together with pale skin, e.g, in general anesthesia, in a sudden critical fall of body

motor adjustments are not found in other mammals. The cat, for instance, is protected against cold passively by its fur and actively only to a small extent by diminishing its surface and by increasing its heat production. Deprived of its fur, its rectal temperature drops rapidly in the cold. At high envi-

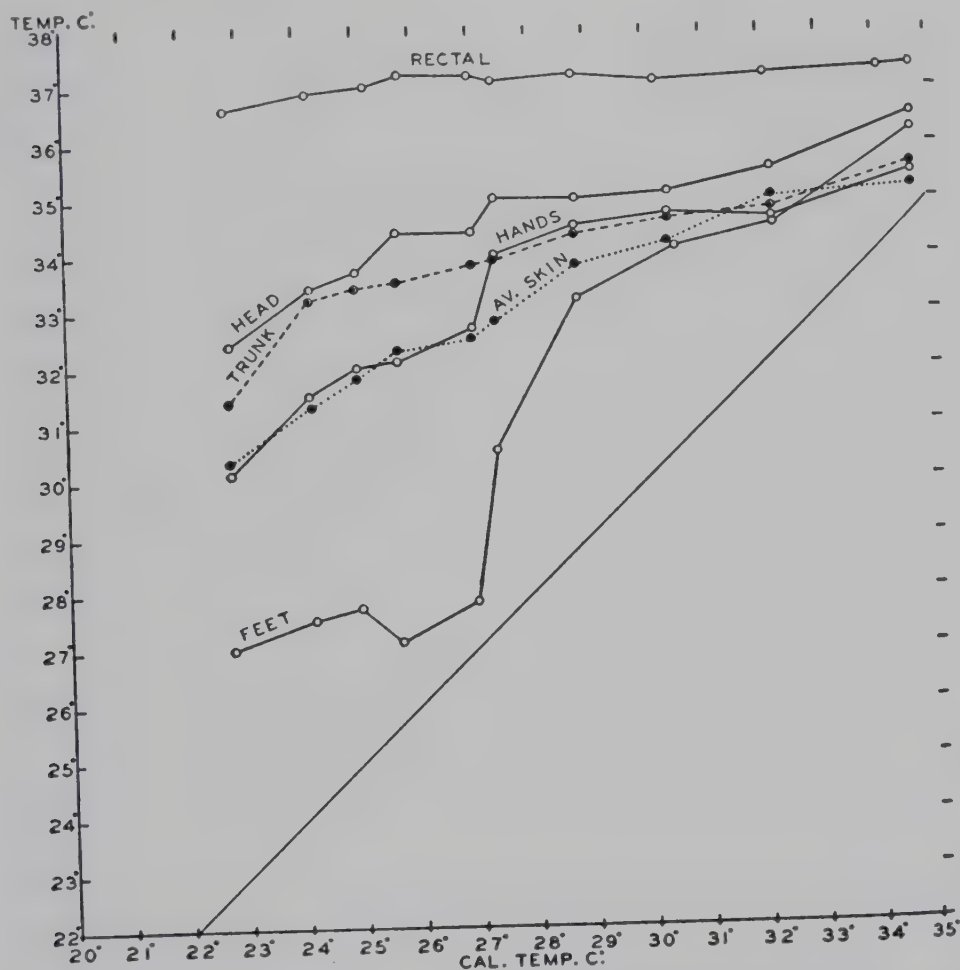


FIG. 9.—Skin and rectal temperatures in normal man during basal experiments in a calorimeter at different environmental temperatures. (For effect of cooling read from right to left.) From Hardy and Du Bois (35). (Reproduced by permission of Reinhold Publishing Corporation.)

temperature of patients with high fever, after administration of antipyretics, and after intake of alcohol. The range of cutaneous blood flow in man is, indeed, remarkably great. The average blood flow in fully vasoconstricted skin is probably less than 15 cc/sq meter/min. During moderately active vasodilatation at a room temperature of 35° C. it is 230 cc/sq meter/min, and during severe exercise 1,200 cc/sq meter/min (37, 80). Comparable thermoregulatory vaso-

ronmental temperatures, in spite of great increase in respiration and of coating of the accessible parts of the fur with saliva, hyperthermia rapidly ensues, with nausea, vomiting, convulsions, and death (62).

Because vasodilatation in response to warmth raises the capillary pressure, it was assumed that there is an increased fluid transudation and an increase in the water content of the skin (4). This would increase thermal conductivity and heat loss. Indeed,

it often appears that the warm skin has more turgor and its surface is smoother, which might be due to a small degree of swelling. Still it is remarkable that such an increase in water content has no influence on insensible water loss. Below the critical temperature of generalized sweat outbreak the insensible

water loss does not increase proportionally with the environmental temperature (69, 71, 49) (p. 233). In acute inflammatory conditions of the skin, when the skin temperature is several degrees higher than normal, insensible water loss is not consistently increased (p. 233).

VIII. FACTORS INFLUENCING SKIN SURFACE TEMPERATURE

A. GENERAL

The discussion in the previous sections has made it clear that there is no such thing as a "normal" temperature of the skin surface, because it varies with a great number of external and internal factors and is different in different parts of the body. Skin temperature measurements have a meaning only if internal and external conditions are well defined. The factors determining the skin temperature have been grouped and are enumerated by Murlin (59) (Table 2).

Many of these factors have been discussed in the previous sections. A few more factors which are important from the point of view of skin physiology and pathology will be considered here briefly.

B. INFLUENCE OF SURFACE PATTERN

Wrinkling of the skin involves increased surface and therefore increased heat loss by radiation and greater coolness of the surface. Smooth skin is warmer. Similarly, on surfaces with sharp curvatures, as seen in lean persons, the temperature is lower than in plump persons with broad curvatures (59).

C. INFLUENCE OF SEBUM

Excess sebum acts like a heat insulator. Under normal circumstances a freshly cleaned skin surface is cooler than a surface covered with sebum. Emulsification of sebum by sweat may promote its removal (41, 42). Thus sweat may exert an additional cooling effect: it increases heat loss by radiation and convection because it removes sebum.

D. REGIONAL DIFFERENCES

Burton's data (15, 16) (Table 1, p. 253) reveal that temperatures on face and trunk

are higher than on extremities. This is so mainly because the surface as related to mass is greater on the extremities. However, Table 2 also shows that under comfortable atmospheric conditions this difference is not great, except on the distal parts of the lower extremities. Benedict (9), examining young girls after a night's rest under the bed cover at room temperatures averaging 18°6 C. (65°5 F.) and at a relative humidity of 70 per cent, found an average skin temperature of 35° C. on the trunk and 30° C. on the feet. But this difference became less when the room temperature was raised to 30° C. (86° F.). In this case the skin temperature of the trunk was 36° C., that of the feet, 33°4 C.

In intertriginous areas, particularly in axillae and groins, the skin temperature is always relatively high because of restricted radiation and convection. Somewhat higher surface temperatures than in adjacent areas were also noted in the antecubital and popliteal fossae (85).

In naked persons the face is usually warmer than the trunk, but clothing either equalizes this difference or reverses it. The forehead was found to have the relatively most constant temperature.

Differences larger than 1° C. on symmetrical sites of distal parts of the extremities indicate peripheral vascular disease (73). For details on thermoregulatory disturbances in these diseases see Sheard (73), Abramson (1), and Richards (64).

The skin temperature is measurably increased over large subcutaneous arteries. A projection of collecting superficial veins carrying cooled blood can be plotted on the skin surface in the form of cool streaks at cool atmospheric temperatures. Regardless

of the great regional differences, many attempts have been made to establish the average skin temperature. The most recent attempt is that of Hensel (39), who registers continuously skin surface temperatures from nine sites, viz., forehead, chest, abdomen, upper arm, dorsum of hand, thigh, leg, and dorsum of foot. He integrates the values by considering how much of the total surface area is represented by each region and calculating how much they contribute accordingly to the average temperature.

E. INFLUENCE OF CLOTHING

Heat loss by radiation and convection is not substantially modified by conventional clothing at moderate temperatures. However, it conveys quite substantial protection beyond the "normal" range of atmospheric temperature in both directions, against excessive cold and excessive heat.

Below 25° C. (77° F.) atmospheric temperature the skin surface temperature is higher than the temperature on the surface of clothes, an indication that heat loss from the skin is retarded (33). In this range the skin surface temperature is higher with than without clothing. For instance, at 22° C. (72° F.), by covering the naked body with clothing, the skin temperature rises from 32.2° C. (90° F.) to 33.9° C. (93° F.). Conversely, in disrobing, there is a decrease in skin surface temperature on the previously covered parts. First the fall is rapid, then it becomes slower. The final temperature is reached in $\frac{1}{2}$ – $1\frac{1}{2}$ hours (73).

The protective action against cold depends on the thickness of the still air which is entrapped in the layers of the clothing, still air being one of the best heat insulators (24). Barring extreme degrees of cold, the type of material—cotton or wool, for instance—is relatively unimportant in this protecting effect. A person not supposed to wear woolen garments can protect himself satisfactorily with several layers of cotton, if we disregard extreme degrees of cold.

Compression of the air layer as it occurs in a man lying in a sleeping bag or wearing tight gloves reduces the thickness of the air

TABLE 2

SUMMARY OF FACTORS INFLUENCING SKIN TEMPERATURE (59)

A. Causes of low skin temperature

I. Internal

a) General

1. Vasoconstriction (from any cause)
2. Moistness of skin (from within or without)
3. Subcutaneous fat (or other poor conductor)
4. Roughness of skin (wrinkles)
5. Sharp curvatures (leanness)
6. Absence of protective covering
7. Cleanliness of skin

b) Local

1. Thick epidermis (calluses)
2. Distance from heart (blood cools on way)
3. Vasodilatation from stagnation
4. Overlarge veins (if extremities are cold)
5. Large surface in relation to mass (fingers)
6. Surfaces which do not radiate toward each other

II. External

1. Low temperature of surrounding walls and air
2. High humidity (with low temperature)
3. Wind or artificial air currents

B. Causes of high skin temperature

I. Internal

a) General

1. Vasodilatation (and increased flow)
2. Dry skin (relative to vapor tension; absent or defective sweat glands)
3. Absence of fat (nearness of large muscular masses and organs)
4. Smooth skin (youthfulness)
5. Broad curvatures (plumpness)
6. Protective covering (poor conductors)
7. Dirt, grime, excess sebum

b) Local

1. Thin epidermis
2. Nearness to heart
3. Local relative vasoconstriction preventing stagnation
4. Overlarge arteries
5. Small surfaces relative to mass (breasts)
6. Surfaces radiating toward each other

II. External

1. High temperature of surrounding objects and air
2. High humidity (with high temperature)
3. Stagnant air (or high wind at high temperature)
4. Insolation (and other radiations)

layer, with a corresponding reduction in protection. On the other hand, too large air spaces, i.e., wider than 4 or 5 mm., make possible convective losses which may become quite high (24).

In arctic regions difficulties arise from vaporization of water from the skin surface. Exercise in dry heavy clothing in the cold causes vaporization from the skin and increased heat loss. The water condenses in the cold intermediate layers of the clothing. Some of the heat produced by the condensation may benefit the body, but most of it is lost to the outer layers of the clothing. When the skin vaporizes water faster than the outer layer, the clothing becomes wet, and insulation will be poor. Under such circumstances rest after exercise is particularly dangerous, because at rest the moisture evaporates from the garment and this causes severe chilling of the resting person. Wet or frozen garments, therefore, must be avoided in severe cold (24).

Clothing is useful against excessive dry heat, as it protects against the direct rays of the sun and the hot air. Above 36° C. (97° F.) air temperature, the temperature on the surface of clothes is higher than on the skin. This indicates retardation of heat penetration from the outside. The skin temperature can be kept considerably lower than that of the clothing when there is evaporative heat loss cooling the skin, as is the case in dry heat. Naturally, it is advantageous to wear white garments, which reflect visible radiation, because this is transformed into heat when absorbed by the skin (p. 264). In severe heat and under strenuous exercise when sweating ability gradually declines (p. 213), this decline is faster in persons clad in army tropical uniforms than in those wearing just broadcloth shorts (34).

In a moist, hot atmosphere the permeability to water vapor is the most important feature of garments, both for protection against overheating and for keeping the skin healthy. Materials impermeable to water vapor will, in the long run, hinder the evaporation, with subsequent heat stasis,

imbibition by the horny layer, and occlusion of sweat-gland pores. Evaporation from the skin is more efficient than evaporation from moist clothing (66).

When the temperature of the air is higher than that of the skin, it is also important to use garments which, while permeable to vapor, are relatively impermeable to moving air, because moving hot air would further increase skin temperature. There are tightly woven fabrics which are hardly permeable to moving air but are as permeable to water vapor as other more loosely woven material. Obviously, they are ideal for the skin in the tropics.

The type of appropriate clothing as a thermal barrier has become the subject of elaborate scientific investigations in the last fifteen years. The respective roles of different properties of the garments, such as thickness, density, resilience, flexibility, air permeability, waterproofness, moisture-absorption capacity, etc., have been studied in detail (61). Unfortunately, however, the influence of different fabrics used for garments in skin diseases has not yet been systematically studied.

Since the work of Gagge, Winslow, and Herrington (33) and Gagge, Burton, and Bazett (32), the effect of clothing on cutaneous heat loss has been expressed semiquantitatively. These authors introduced the terms "Met" and "Clo." One "Met" is the heat production of a man sitting quietly in a chair in a room at ordinary temperature. One "Clo" is the protective value of the ordinary clothing that keeps him comfortable under these conditions. A heavy suit used for extreme cold has a value of 3 Clo. It will protect a man from losing body heat in an environment of 0° C. (32° F.) if he is resting and producing only 1 Met. If he is walking and increasing his heat production three times, to 3 Mets, he will still remain fairly comfortable at 0° C. If a man is sleeping and producing only 0.8 Met in a tent at -29° C. (-20° F.), a sleeping bag would be needed that provides 11 Clo, and this is impossible to secure (24). Indices of comfort have been discussed by Yaglou (87).

IX. HEAT REGULATION IN DISEASED SKIN

Occlusion of venous circulation is immediately followed by a drop in skin temperature in the area of venous stasis. In stasic conditions the regulatory effect of blood flow is markedly decreased, and there is a greater tendency toward equalization between the temperature of the skin and that of the atmosphere. The "dryness" of the skin in stasic dermatitis, often associated with itching, might have something to do with decreased blood flow, decreased sweating, and decreased sebaceous-gland activity.

In generalized erythematous inflammations of the skin, thermoregulation is disturbed by several mechanisms. In true acute inflammatory processes the dilatation of the superficial minute vessels is associated with a true increased blood flow, and therefore the temperature of the skin is abnormally high. The skin surface temperature in acute solar dermatitis, for instance, was found to be 8° C. higher than that of the surrounding normal skin (29). Hence heat loss by radiation and convection is greatly increased. This is the reason for chills without fever in patients with generalized acute dermatitides. If these patients are kept warm, the chills can be suppressed. The ability of the minute vessels to contract is greatly reduced in this situation. Early experiments by me and my colleagues indicated that higher concentrations of epinephrine are required to cause visible contraction of the minute vessels in acute dermatitides than in normal skin (70) (p. 80). In generalized acute dermatitides the skin is unable to respond with general vasoconstriction to low environmental temperatures. Under such circumstances

the heat balance must be maintained by increased heat production (food intake, exercise, shivering) or by raising the atmospheric temperature.

In subacute and chronic generalized erythematous inflammatory processes the situation is different because in these conditions vasodilatation does not necessarily imply increased blood flow and warm skin. The best example is chronic exfoliative dermatitis. In most cases the skin temperature is not greatly elevated. However, in these cases evaporative heat loss is greatly enhanced, up to tenfold (29). This is due partly to accelerated keratinization (p. 234) and partly to increased permeability of the skin to water from the inside to the outside by virtue of parakeratosis (29). This is the reason that these patients, too, shiver easily and that their fluid intake is greatly increased. In patients with generalized erythroderma Mahli (55) found high skin temperatures, which, however, were dampened by the greatly increased insensible water loss. The increased blood flow to the skin did not diminish in a moderately cool environment. When cooled energetically, the body temperature fell almost as in a dead body, in accordance with Newton's law of cooling. When cooled moderately, these patients reacted with chill and increased basal metabolic rate. This increase in metabolism was secondary to cooling and was much less than would have been expected from the increased insensible water loss. It was quite obvious that with diseased skin there is no parallelism between insensible water loss and basal metabolic rate (p. 239).

X. LOCAL EFFECTS OF COLD

Moderate cold stimuli cause paling of the skin. After the cold stimulus has ceased, a reactive arterial vasodilatation follows. It is because of this reaction that in most acute inflammatory conditions the application of ice or ice-cold water is not advisable. Although the primary vasoconstriction gives great

subjective relief and has antiphlogistic action objectively, the subsequent hyperemia makes the situation worse than it was before the cold application. Burckhardt (14) has dealt with this reaction and found that re-warming of cold hands is much slower in women between menarche and menopause

than in men of comparable age. However, during pregnancy rewarming is greatly accelerated.

Lewis (52) found that even if exposure to cold is maintained, there is a reactive hyperemia to cold and that this reaction is a rhythmical phenomenon. If a finger is kept in ice water and the skin temperature falls to near 0° C., there is a spontaneous rewarming, with a maximum rise of about 10° C. after 15 minutes. Later, the skin temperature falls again and rises again in rhythmical periods. The waves may repeat themselves many times if the finger is kept in ice water (Fig. 10). Lewis presented evidence that the periodic vasodilatation is due to periodic axon-reflex impulses. More recently, however, Kramer and Schulze (48) demonstrated a periodicity of central nervous system impulses which are in the background of the axon-reflex rhythmicity. They found that rewarming is due to dilatation of arterioles and arteriovenous anastomoses. They were able to prolong the vasodilatory periods by administration of hot fluids and by intake of alcohol.

Extensive studies in this field were done by Blaisdell (11). He found that the intermittent vasodilatation of the fingers in the cold occurred not only when the body as a whole was warm but also when the body was thoroughly chilled. Thus the blood flow to the fingers did not depend on the thermoregulatory "needs" of the body as a whole but was a rather independent local reaction. However, the degree of cold necessary to evoke the cyclic hyperemic response was less, the warmer the body. He interpreted the rhythmical phenomenon as being due to changing of the spatial thermal gradient. A critical temperature difference between the warm blood and the surface of the cooled finger sets up a steep thermal gradient which stimulates the axon-reflex receptors to initiate local reflex vasodilatation; when warm blood flows through, the gradient decreases, vasodilatation subsides, the finger cools, and vasoconstriction ensues. He found that this hyperemia has a definite local protective value but does not necessarily inhibit frost-

bite, because frostbite sets in at the initial cooling period.

Cold stimuli of long duration bring about a livid discoloration as the result of paresis in the venous limbs of the capillaries. Cold stimuli of extreme intensity are followed by the appearance of a vivid red color, which is due to paresis of arterioles; in spite of arteriolar dilatation, this condition is not connected with increased blood flow, the temperature of the skin does not rise, and the heat loss is not increased. Such paralytic arterial hyperemia can persist when the metabolism of the tissue is considerably diminished, so that the oxyhemoglobin of the blood remains unused. This condition represents the first grade or the erythematous phase of freezing or congelation. It is followed by stasis in the venous system and passive hyperemia, later on by edema. Congelation of the second and third degrees (blister formation, hemorrhage, necrosis) is due to severe cellular damage in the vessel walls and in the epidermis.

In true congelation those parts of the body surface are primarily endangered which carry the brunt of vasomotor heat regulation, namely, the acrae. The regulative capacity of fingers, toes, hands, feet, ears, and nose is potentiated by the great number of arteriovenous anastomoses in these areas, and it appears that these parts of the body are, so to speak, sacrificed to maintain body temperature.

In contrast to congelation, chilblain (pernio) does not develop at extreme degrees of cold but at temperatures around and above freezing point exclusively in individuals with constitutionally insufficient capillary circulation in fingers and toes. These individuals, always having "cold feet and cold hands," are the victims of acrocyanosis and swellings in moderately cold weather and develop nodular lesions around damaged blood vessels (50).

"Immersion foot" or "trench foot" is a pathological state of the vessels and tissues of the feet brought about by prolonged chilling of the feet by cold water. Apparently, cold (not necessarily freezing) temperatures

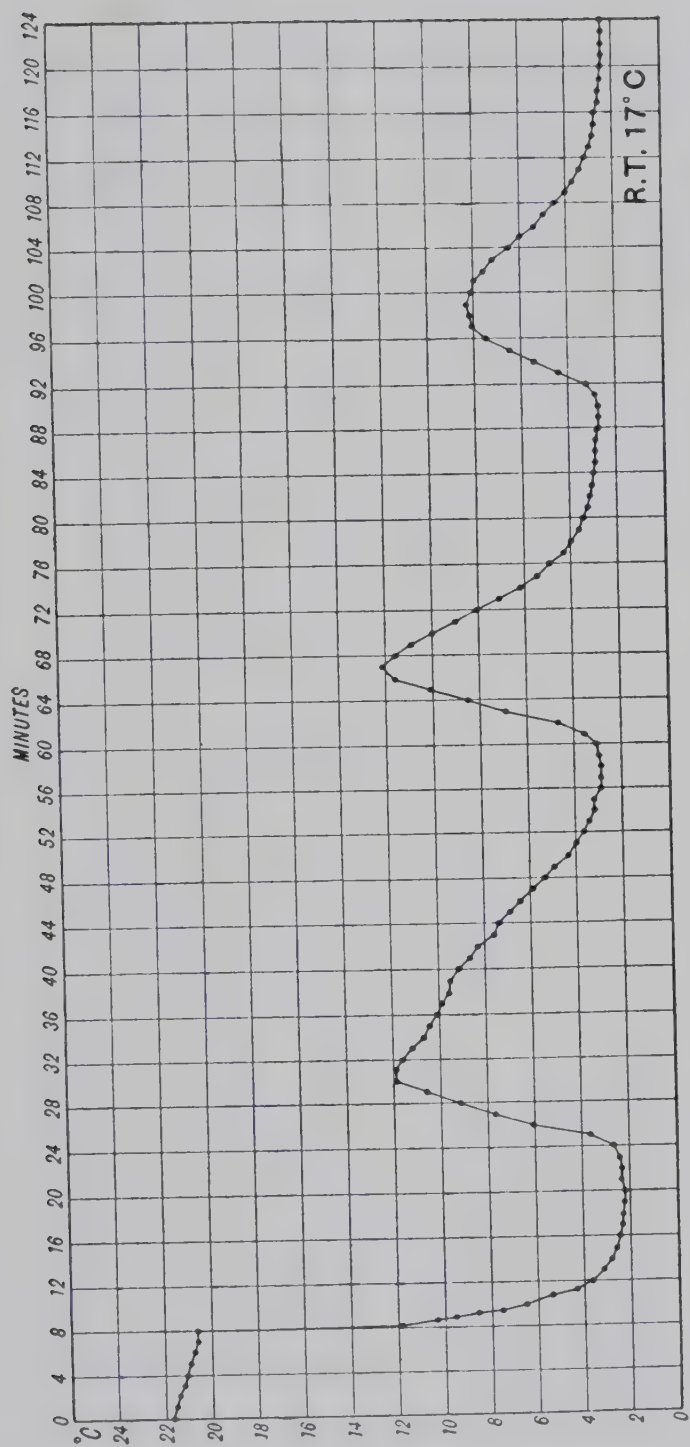


FIG. 10.—Curve of temperature from right second finger during immersion in crushed ice, showing very prolonged and large oscillations of temperature. Water unstirred. From Lewis (52). (Reproduced by permission of Clinical Science.)

and moisture act synergistically in developing this severe condition (10). The immediate reaction to cold is vasoconstriction, and, according to the duration and severity of the exposure, all degrees of capillary damage may result. If after the exposure to cold water the feet are brought rather suddenly to normal temperatures, persistent reactive hyperemia (p. 261) results, and there is swelling, blistering, ulceration, and gan-

grene, because in the vasodilatory phase capillary damage permits leakage of plasma into the tissues. Thrombosis often seriously complicates the situation (12). After 6–10 weeks the hyperemic stage merges into one in which the feet are pale, cold, and very sensitive to cold. In the chronic stage the disease is characterized by severe disturbances in the regulation of blood flow (63).

XI. PENETRATION OF THERMAL STIMULI

By simple contact with warm objects, only the most superficial layers of the skin are heated. If heating pads or hot-water bottles have any effect on the circulation of underlying deep-seated organs, this must be achieved by local reflex action. The epidermis can be scalded, but the temperature in

ture is produced by a carbon-arc lamp with its rich visible radiation, the temperature in the lower corium is as high as 44° C. (111° F.). It may even happen that deep layers become warmer than the surface when the skin is irradiated with visible rays, because of greater absorption in the depth (Table 3).

TABLE 3
PENETRATION OF HEAT THROUGH THE SKIN
FROM DIFFERENT SOURCES (76)

Equal Energies of:	Surface Temperature (° F.)	Temperature 3 Mm. be- low Surface (° F.)
Visible rays.....	106.7	109.6
Short-wave infrared.....	111.5	107.1
Long-wave infrared.....	110.7	104.9

Infrared rays are absorbed superficially and cause heat damage wherever they are absorbed. Visible rays penetrate deeper and transform into heat after they have been absorbed in deeper layers.

High-frequency currents (“diathermy”) warm the tissues all along their pathway, according to their intensity and the heat capacity of the tissues. All along the current the electric energy is transformed into heat. This is Joule’s heat, which is proportional to the square of the electric intensity. The difference in effects of contact heating and heating by ambient air and radiant heat was discussed on page 252.

superficial blood vessels does not change. Similarly, long-wave infrared rays do not penetrate to any appreciable depth.

In contrast, short-wave infrared rays and particularly visible rays, mainly red and yellow, warm the skin throughout its entire thickness without damaging the surface (76–79). For example, if the surface is warmed by a dark hot iron plate (long-wave infrared rays) to 45° C. (113° F.), the temperature in the lower corium is 40° C. (103.6° F.). But if the same surface tempera-

Application of cold objects to the skin carries cold deeper down than warm objects carry heat. The deep corium can be cooled quite considerably by ice compresses. Although the skin with constricted vessels is a poor conductor, the fact that there is no circulating blood to bring heat from within to the surface makes such effective cooling possible.

XII. CHANGES IN SWEAT SECRETION DURING ACCLIMATIZATION TO HIGH ENVIRONMENTAL TEMPERATURES

In acclimatization to high temperatures, cardiovascular adjustments as studied by Dill (22), Bazett (72), Adolph (2, 3), Keys (82), and others are of paramount importance. In addition, however, vitally important adjustments take place also in the sweating mechanism.

In subjects not acclimatized to high environmental temperatures the rectal temperature rises considerably before sweating sets in. With successive day-by-day exposures, *reflex sweating will start at progressively lower rectal temperatures*. This conditioning of reflex sweating is particularly rapid in the first 3-4 days (65, 68, 51). With further exposures, a new adjustment takes place: *at a given rectal temperature the sweat rate increases* (51). This is the principal adaptive mechanism. In the nonacclimatized individual the skin temperature rises considerably, owing to radiation from the outside, causing the thermal gradient from the inside of the body to the outside to decrease. Large peripheral blood flow (an attempt to increase heat radiation outward) causes inadequate blood flow in internal organs. The circulation becomes overtaxed and unstable (28). With increased evaporative cooling in the acclimatized individual, these difficulties are largely overcome. Cooling of the surface widens the internal thermal gradient, and a relatively small rate of blood flow to the periphery suffices to transfer deep heat to the periphery.

A third important adjustment is a significant *reduction in the salt concentration of sweat*. This occurs after a few days of acclimatization (51). Whereas Johnson *et al.* (47) thought that the lowered chloride concentration was an indirect effect in acclimatization secondary to better cooling of the skin (p. 213) and to lower rectal temperatures, Robinson *et al.* (67) found that even if rectal temperatures, skin temperatures, and sweating rates are constant, acclimatization invariably leads to a decrease in

chloride secretion. The mechanism of this adaptation phenomenon, first observed by Talbott, Edwards, Dill, and Drastich (81), was clarified by the brilliant work of Conn (19, 20, 18).

Conn, first studying the effect of desoxycorticosterone (DOCA), found that, whereas urinary sodium chloride excretion is depressed by this hormone for only a few days and then rebounds to much higher values than normal, the sodium and chloride content of sweat continues to be low under the influence of this salt-saving steroid as long as it is given. When administration of DOCA is discontinued, there is a rebound effect in sweat, too. When adrenocorticotrophic hormone (ACTH) is given, the sweat chloride concentration is similarly depressed. The effect is different because ACTH also causes a negative nitrogen balance by virtue of its catabolic effect on proteins. This makes the effect of ACTH identical with that of natural acclimatization, in which the nitrogen balance is negative as long as the adjustment of sodium chloride secretion is made. With DOCA there is rather a tendency to nitrogen retention, probably because of pituitary suppression.

Conn came to the conclusion that natural acclimatization to strenuous work in hot environment is a pituitary-adrenal effect, corresponding to a chronic stress situation. In such a situation there is continuous activity of salt-saving steroids, with a gradual disappearance of the stimulating action on protein decomposition. In acute stress (for instance, if salt is withdrawn in strenuous work) the negative nitrogen balance persists. According to Conn, the chloride concentration of sweat may be used as an index of activity of the salt-saving steroids.

Locke *et al.* (54) called attention to the difficulties which arise from the great variability in the salt concentration of sweat under "normal" conditions. This makes it, according to Locke, very difficult to judge

the activity of DOCA-like steroids simply from the salt concentration of sweat. The two main factors on which the sodium and chloride concentration of sweat depends are the rate of sweating and the skin temperature (see p. 213). The sweat chloride/sweating-rate index of Locke *et al.* (see p. 213), if expressed in certain mathematical terms, gives true "normal," individually constant values. The role of skin temperature and of the intensity of the thermal stimulus are also taken care of in this formula.⁷ The administration of ACTH or of DOCA is accompanied by a striking reduction in these index values, whereas no such effect is achieved by cortisone, methyltestosterone, or progesterone. These latter hormones rather have the tendency to raise the index, probably because of pituitary suppression.

The index is conspicuously high in adrenal insufficiency. These high values can be approximated in normals if excessive amounts of salt are given. The real characteristic feature of adrenal insufficiency is that there is no adjustment of the index with changing conditions. Whereas in nor-

mals the sweat chloride adjusts itself to changes in sodium chloride intake, to repeated exposures to heat, and to changes in potassium content of the diet, such adjustments are lacking in adrenal insufficiency.

Thus the decrease in sodium chloride concentration of sweat in acclimatization is clearly an endocrine function. It is, however, remarkable that not only the concentration of sodium chloride but also that of lactic acid decreases considerably in acclimatization (p. 211). It is difficult to interpret this change as a reaction to chronic stress. It is assumed that part of the lactate in sweat originates from glycogen in the sweat gland (p. 210). Steroid hormones having a glycconeogenic effect would promote rather than suppress the breakdown of glycogen.

The saving of sodium chloride by acclimatization is indeed quite substantial. The chloride concentration decreases by 60–70 per cent. If an unacclimatized man loses 4 gm NaCl/l, or, with 8 liters sweat daily, a total of 32 gm., this amount is reduced after acclimatization to 9.5–13 gm. (18).

7. Chloride concentration is expressed in mEq/l, sweating rate in ml/hr/0.1 sq meter surface.

BIBLIOGRAPHY

1. ABRAMSON, D. I. Vascular responses in the extremities of man in health and disease. Chicago: University of Chicago Press, 1944.
2. ADOLPH, E. F. Heat exchange of man in the desert, *Am. J. Physiol.*, **123**:486–99, 1938.
3. ———. Physiology of man in the desert. New York: Interscience Publishers, 1947.
4. BAZETT, H. C. The effect of temperature on the skin: a review, *J. Invest. Dermat.*, **1**: 413–25, 1938.
5. ———. Blood temperature and its control, *Am. J. M. Sc.*, **218**:483–92, 1949.
6. ———. Theory of reflex controls to explain regulation of body temperature at rest and during exercise, *J. Appl. Physiol.*, **4**:245–62, 1951.
7. BAZETT, H. C.; LOVE, L.; NEWTON, M.; EISENBERGH, L.; DAY, R.; and FORSTER, R., II. Temperature changes in blood flowing in arteries and veins in man, *J. Appl. Physiol.*, **1**:3–19, 1948.
8. BAZETT, H. C., and MCGLONE, B. Temperature gradients in the tissues in man, *Am. J. Physiol.*, **82**:415–51, 1927.
9. BENEDICT, F. G. Skin temperature and heat loss, *Proc. Nat. Acad. Sc.*, **11**:549, 1925.
10. BEST, C. H., and TAYLOR, N. B. The physiological basis of medical practice. Baltimore: Williams & Wilkins Co., 1950.
11. BLAISDELL, R. K. Cold-induced vasodilatation. Environmental Protection Section, Rept. No. 177. Quartermaster Climatic Laboratory, Research and Development Branch, Military Planning Division, Office of the Quartermaster General, U.S. Army, September, 1951.
12. BLOCK, M. Genesis of the gangrenous and reparative processes in trench foot, *Arch. Path.*, **46**:1–34, 1948.
13. BOHNENKAMP, H., and ERNST, H. W. Untersuchungen zu den Grundlagen des Energie- und Stoffwechsels; über die Strahlungs-

- verluste des Menschen; die Strahlungsmessung im absoluten Energiemass, Arch. f. d. ges. Physiol., **228**:63-78, 1931.
14. BURCKHARDT, W., and SZADURSKI, J. A. R. Die Wiederwärmung der Haut nach einem kalten Handbad beim Gesunden, Dermatologica, **97** (suppl.): 23-29, 1948.
15. BURTON, A. C. Application of theory of heat flow to study of energy metabolism, J. Nutrition, **7**:497-533, 1934.
16. ———. Operating characteristics of the human thermoregulatory mechanism. In: Temperature: its measurement and control in science and industry, pp. 522-28. New York: Reinhold Publishing Corp., 1941.
17. BÜTTNER, K. Über die Wärmestrahlung und die Reflexionseigenschaften der menschlichen Haut, Strahlentherapie, **58**:345-60, 1937.
18. CONN, J. W. The mechanism of acclimatization to heat. In: Advances in internal medicine, **3**:373-93. New York: Interscience Publishers, Inc., 1949.
19. ———. Electrolyte composition of sweat; clinical implications as an index of adrenal cortical function, Arch. Int. Med., **83**:416-28, 1949.
20. CONN, J. W.; LOUIS, L. H.; JOHNSON, M. W.; and JOHNSON, B. Electrolyte content of thermal sweat as an index of adrenal cortical function, J. Clin. Investigation, **27**:529-30, 1948.
21. CRAIG, W. McK.; HORTON, B. T.; and SHEARD, C. Thermal changes in peripheral vascular disease during sympathetic ganglionectomy under general anesthesia, J. Clin. Investigation, **12**:573-81, 1933.
22. DILL, D. B. Life, heat, and altitude. Cambridge: Harvard University Press, 1938.
23. DU BOIS, E. F. The mechanism of heat loss and temperature regulation. (Lane medical lectures.) Stanford, Calif.: Stanford University Press, 1937.
24. ———. Fever and the regulation of body temperature. Springfield, Ill.: Charles C Thomas, 1948.
25. DU BOIS, E. F., and HARDY, J. D. Relationship of humidity to evaporation of sweat, Science, **87**:430, 1938.
26. EICHNA, L. W.; BEAN, W. B.; ASHE, W. F.; and NELSON, N. Performance in relation to environmental temperature; reactions of normal young men to hot, humid (simulated jungle) environment, Bull. Johns Hopkins Hosp., **76**:25-58, 1945.
27. EICHNA, L. W.; BERGER, A. R.; RADER, B.; and BECKER, W. H. Comparison of intracardiac and intramuscular temperatures with rectal temperatures in man, J. Clin. Investigation, **30**:353-59, 1951.
28. EICHNA, L. W.; PARK, C. R.; NELSON, N.; HORVATH, S. M.; and PALMES, E. D. Thermal regulation during acclimatization in a hot, dry (desert type) environment, Am. J. Physiol., **163**:585-97, 1950.
29. FELSHER, Z., and ROTHMAN, S. The insensible perspiration of the skin in hyperkeratotic conditions, J. Invest. Dermat., **6**:271-78, 1945.
30. FOGED, J. Die normale Hauttemperatur, Skandinav. Arch. f. Physiol., **64**:251-78, 1932.
31. GAGGE, A. P. A new physiological variable associated with sensible and insensible perspiration, Am. J. Physiol., **120**:277-87, 1937.
32. GAGGE, A. P.; BURTON, A. C.; and BAZETT, H. C. A practical system of units for the description of heat exchange of man with his environment, Science, **94**:428-30, 1941.
33. GAGGE, A. P.; WINSLOW, C.-E. A.; and HERRINGTON, L. P. The influence of clothing on the physiological reactions of the human body to varying environmental temperatures, Am. J. Physiol., **124**:30-50, 1938.
34. GERKING, S. D., and ROBINSON, S. Decline in the rate of sweating of men working in extreme heat, Am. J. Physiol., **147**:370-78, 1946.
35. HARDY, J. D., and DU BOIS, E. F. The significance of the average temperature of the skin. In: Temperature: its measurement and control in science and industry, pp. 537-43. New York: Reinhold Publishing Corp., 1941.
36. HARDY, J. D., and MUSCHENHEIM, C. Radiation of heat from human body; emission, reflection and transmission of infra-red radiation by human skin, J. Clin. Investigation, **13**:817-31, 1934.
37. HARDY, J. D., and SODERSTROM, G. F. Heat loss from the nude body and peripheral blood flow at temperature of 22° C. to 35° C., J. Nutrition, **16**:439-510, 1938.
38. HENRIQUES, F. C., JR., and MORITZ, R. A. Studies of thermal injury. I. The conduction of heat to and through skin and the temperature attained therein. A theoretical and experimental investigation, Am. J. Path., **23**:531-49, 1947.

39. HENSEL, H. Ein Gerät zur fortlaufenden Registrierung der integralen Hauttemperatur und der Hauttemperaturen einzelner Körperstellen ("Thermointegralschreiber"), *Arch. f. d. ges. Physiol.*, **251**:388-97, 1949.
40. ———. Die intracutane Temperaturbewegung bei Einwirkung äusserer Temperaturreize, *ibid.*, **252**:146-64, 1950.
41. HERRMANN, F., and PROSE, P. H. Studies on the ether-soluble substances on the skin. I. Quantity and "replacement sum," *J. Invest. Dermat.*, **16**:217-30, 1951.
42. HERRMANN, F.; PROSE, P. H.; and SULZBERGER, M. B. Studies on sweating. V. Studies of quantity and distribution of thermogenic sweat delivery to the skin, *J. Invest. Dermat.*, **18**:71-86, 1952.
43. HEYNINGEN, R. VAN, and WIENER, J. S. Comparison of arm and general body sweat, *J. Physiol.*, **112**:13P, 1950.
44. HORTON, B. T.; SHEARD, C.; and ROTH, G. M. Vasomotor regulation of temperatures of extremities in health and disease, *J. M. Soc. New Jersey*, **37**:311-23, 1940.
45. IPSEN, J. *Hauttemperaturen*. Leipzig: Georg Thieme, 1936.
46. IVY, A. C. What is normal or normality, *Quart. Bull., Northwestern Univ. M. School*, **18**:22-32, 1944.
47. JOHNSON, R. E.; PITTS, G. C.; and CONSO LAZIO, F. C. Factors influencing chloride concentration in human sweat, *Am. J. Physiol.*, **141**:575-89, 1944.
48. KRAMER, K., and SCHULZE, W. Die Kältedilatation der Hautgefässe, *Arch. f. d. ges. Physiol.*, **250**:141, 170, 1948.
49. KUNO, Y. *The physiology of human perspiration*. London: J. & A. Churchill, Ltd., 1934.
50. KYRLE, J. Über Pathologie und Klinik der durch Kältewirkung in Beruf und Arbeit, einschliesslich der Kriegsarbeit hervorgerufenen Aenderungen der Haut, nebst kurzen Bemerkungen über Prophylaxe und Therapie derselben. In: OPPENHEIM, RILLE, and ULLMAN, *Die Schädigungen der Haut*, **1**: 73-96. Leipzig: Leopold Voss, 1932.
51. LADELL, W. S. S. Thermal sweating, *Brit. M. Bull.*, **3**:175-79, 1945.
52. LEWIS, T. Observations upon the reactions of the vessels of the human skin to cold, *Heart*, **15**:177-208, 1929-31.
53. LIESE, W. *Hauttemperaturmessungen am ruhenden und arbeitenden Menschen unter dem Einfluss schwacher Luftströme. Zugleich ein Beitrag zur Katathermometrie*, *Arch. f. Hyg.*, **104**:24-42, 1930.
54. LOCKE, E.; TALBOT, N. B.; JONES, H. S.; and WORCESTER, L. Studies on the combined use of measurement of sweat electrolyte composition and rate of sweating as an index of adrenal cortical activity, *J. Clin. Investigation*, **30**:325-37, 1951.
55. MAHLI, W. H. Influence of generalized inflammation of the skin on the surface temperature and on the water, *Dermatologica*, **104**:19-43, 1952.
56. MEYER, H. H. *Theorie des Fiebers und seiner Behandlung*, *Verhandl. d. Kong. f. inn. Med.*, **30**:15-25, 1913.
57. MOLE, R. H. The relative humidity of the skin, *J. Physiol.*, **107**:399-411, 1948.
58. MORITZ, A. R., and HENRIQUES, F. C., JR. Studies in thermal injury. II. The relative importance of time and surface temperature in the causation of cutaneous burns, *Am. J. Path.*, **23**:695-720, 1947.
59. MURLIN, J. R. Skin temperature: its measurement and significance for energy metabolism, *Ergebn. d. Physiol.*, **42**:153-227, 1939.
60. NEUROTH, G. Die Hauttemperatur im Dienste der Wärmeregulation, *Arch. f. d. ges. Physiol.*, **250**:396-413, 1948.
61. NEWBURGH, L. H. (ed.). *Physiology of heat regulation and the science of clothing*. Philadelphia: W. B. Saunders Co., 1949.
62. ORONTY, L. R. Heat loss and heat production of cats at different environmental temperature, *Federation Proc.*, **8**:128-29, 1949.
63. REDISCH, W.; BRANDMAN, O.; and RAINONE, S. Chronic trench foot: a study of 100 cases, *Ann. Int. Med.*, **34**:1163-68, 1951.
64. RICHARDS, R. L. *The peripheral circulation in health and disease*. Baltimore: Williams & Wilkins Co., 1946.
65. ROBINSON, S.; DILL, D. B.; WILSON, J. W.; and NIELSEN, M. Adaptations of white men and Negroes to prolonged work in heat, *Am. J. Trop. Med.*, **21**:261-87, 1941.
66. ROBINSON, S., and GERKING, S. D. Thermal balance of men working in severe heat, *Am. J. Physiol.*, **149**:476-88, 1947.
67. ROBINSON, S.; GERKING, S. D.; TURRELL, E. S.; and KINCAID, R. K. Effect of skin temperature on salt concentration of sweat, *J. Appl. Physiol.*, **2**:654-62, 1950.
68. ROBINSON, S.; TURRELL, E. S.; BELDING, A. S.; and HORVATH, S. M. Rapid acclimatization

- zation to work in hot climates, *Am. J. Physiol.*, **140**:168-76, 1945.
69. ROTHMAN, S. Über den Einfluss einiger dermatotherapeutischer Grundsubstanzen auf die insensible Wasserabgabe der Haut, *Arch. f. Dermat. u. Syph.*, **131**:549-67, 1921.
70. ———. Unpublished experiments, 1923.
71. ROTHMAN, S., and SCHAAF, F. Chemie der Haut. *In*: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1/2**:161-377. Berlin: J. Springer, 1929.
72. SCOTT, J. C.; BAZETT, H. C.; and MACKIE, G. C. Climatic effects on cardiac output and circulation in man, *Am. J. Physiol.*, **129**: 102-22, 1940.
73. SHEARD, C. Temperature of skin and thermal regulation of the body. *In*: O. GLASSER, Medical physics, **1**:1523-55. Chicago: Year Book Publishers, 1944.
74. SHEARD, C.; RYNEARSON, E. H.; and CRAIG, W. McK. Effects of environmental temperature, anesthesia and lumbar sympathetic ganglionectomy on temperatures of extremities of animals, *J. Clin. Investigation*, **11**:183-93, 1932.
75. SHEARD, C.; WILLIAMS, M. M. D.; and HORTON, B. T. Skin temperatures of the extremities under various environmental and physiological conditions. *In*: Temperature: its measurement and control in science and industry, pp. 557-70. New York: Reinhold Publishing Corp., 1941.
76. SONNE, C. The mode of action of the universal light bath. 5. Visible and invisible heat rays, *Acta med. Scandinav.*, **54**:374-83, 1921.
77. ———. A thermo-needle with inset regulative heating apparatus, together with some measurements of cutaneous, subcutaneous, and intramuscular heat endurance, *Acta radiol.*, **2**:187-93, 1923.
78. ———. Physiologische und therapeutische Wirkungen des künstlichen Lichtes, *Strahlentherapie*, **20**:829-42, 1925.
79. ———. Über einige physiologische Wirkungen der leuchtenden Strahlungen, *ibid.*, **21**:148-61, 1926.
80. SPEALMAN, C. R. Physiologic adjustments to cold. *In*: NEWBURGH, Physiology of heat regulation and the science of clothing, pp. 232-39. Philadelphia: W. B. Saunders Co., 1949.
81. TALBOTT, J. H.; EDWARDS, H. T.; DILL, D. B.; and DRASTICH, L. Physiological responses to high environmental temperature, *Am. J. Trop. Med.*, **13**:381, 397, 1933.
82. TAYLOR, H. L.; HENSCHER, A. F.; and KEYS, A. Cardiovascular adjustments of man in rest and work during exposure to dry heat, *Am. J. Physiol.*, **139**:583-91, 1943.
83. THAUER, R. Der Mechanismus der Wärmeregulation, *Ergebn. d. Physiol.*, **41**:607-805, 1939.
84. UNNA, P. G. Über die insensible Perspiration der Haut, *Verhandl. d. Kong. f. inn. Med.*, **9**:230-51, 1890.
85. WILLIAMS, D. H. Skin temperature reaction to histamine in atopic dermatitis (disseminated neurodermatitis), *J. Invest. Dermat.*, **1**:119-29, 1938.
86. WINSLOW, C.; HERRINGTON, L. P.; and GAGGE, A. P. Physiological reactions of the human body to various atmospheric humidities, *Am. J. Physiol.*, **120**:288-99, 1937.
87. YAGLOU, C. P. Indices of comfort. *In*: NEWBURGH, Physiology of heat regulation and the science of clothing, pp. 277-87. Philadelphia: W. B. Saunders Co., 1949.

CHAPTER 11

The Sweat-Retention Syndrome

I. THE EFFECT OF HOT, HUMID CLIMATE	270
II. THE EFFECT OF INJURY	277
III. REPERCUSSIONS ON THERMOREGULATION	281

I. THE EFFECT OF HOT, HUMID CLIMATE

MODERN physiologists, when dealing with profuse eccrine sweating and its consequences, have been preoccupied with one vital aspect, namely, the “heat-exhaustion syndrome” as it appears in consequence of excessive loss of salt and water. During the second World War a new, but not less important, phenomenon—the sweat-retention syndrome—was discovered.

This syndrome was first observed among troops serving in hot, humid climates. Normal healthy young men, sweating normally for a while, suddenly or gradually lost their ability to sweat on the trunk and limbs and became moderately hyperthermic, the average body temperature being 100° F. They felt well at rest, but, on even moderate exercise in the heat, they became rapidly exhausted and suffered from headache, palpitation, and dyspnea. In such attacks the pulse rate was accelerated up to 160 beats per minute and respiration up to 50 per minute (11). Some had inflammatory skin eruptions resembling “prickly heat”; but in others nothing could be seen on the skin. As soon as the men were taken to a cooler environment and given rest, they recovered and after varying periods of time regained their sweating ability. The syndrome was termed “thermogenic anhidrosis” by Wolkin *et al.* (30), “heat exhaustion, type II” by Ladell *et al.* (4), “tropical anidrotic asthe-

nia” by Allen and O’Brien (1), and “sweat retention syndrome” by Sulzberger *et al.* (23).

Originally it was thought that this syndrome represented some kind of exhaustion of the sweating mechanism. But O’Brien’s (1, 10, 11, 12) and Sulzberger’s (20, 25, 26, 23, 24, 22, 3) basic work soon elucidated the situation as resulting from a closure of the sweat pores by horny plugs which hinder the outpouring and evaporation of normally secreted sweat. This mechanical type of closure not only profoundly disturbs heat regulation as a whole but also can have a formidable effect on the skin proper. The retained sweat breaks through the wall of the maximally dilated ducts and, as a highly undesirable foreign material, disturbs most seriously the normal life-processes of the skin, mechanically and chemically. Because of these far-reaching consequences, the phenomenon has drawn the attention of several groups of investigative dermatologists during the last decade. In a long series of brilliant pieces of work a great number of new facts were discovered, the most important being that plugging of the sweat pores is an extremely common event, by no means restricted to tropical environments.

O’Brien (10) has followed closely the structural changes of the poral closure and gives the following account. The terminal

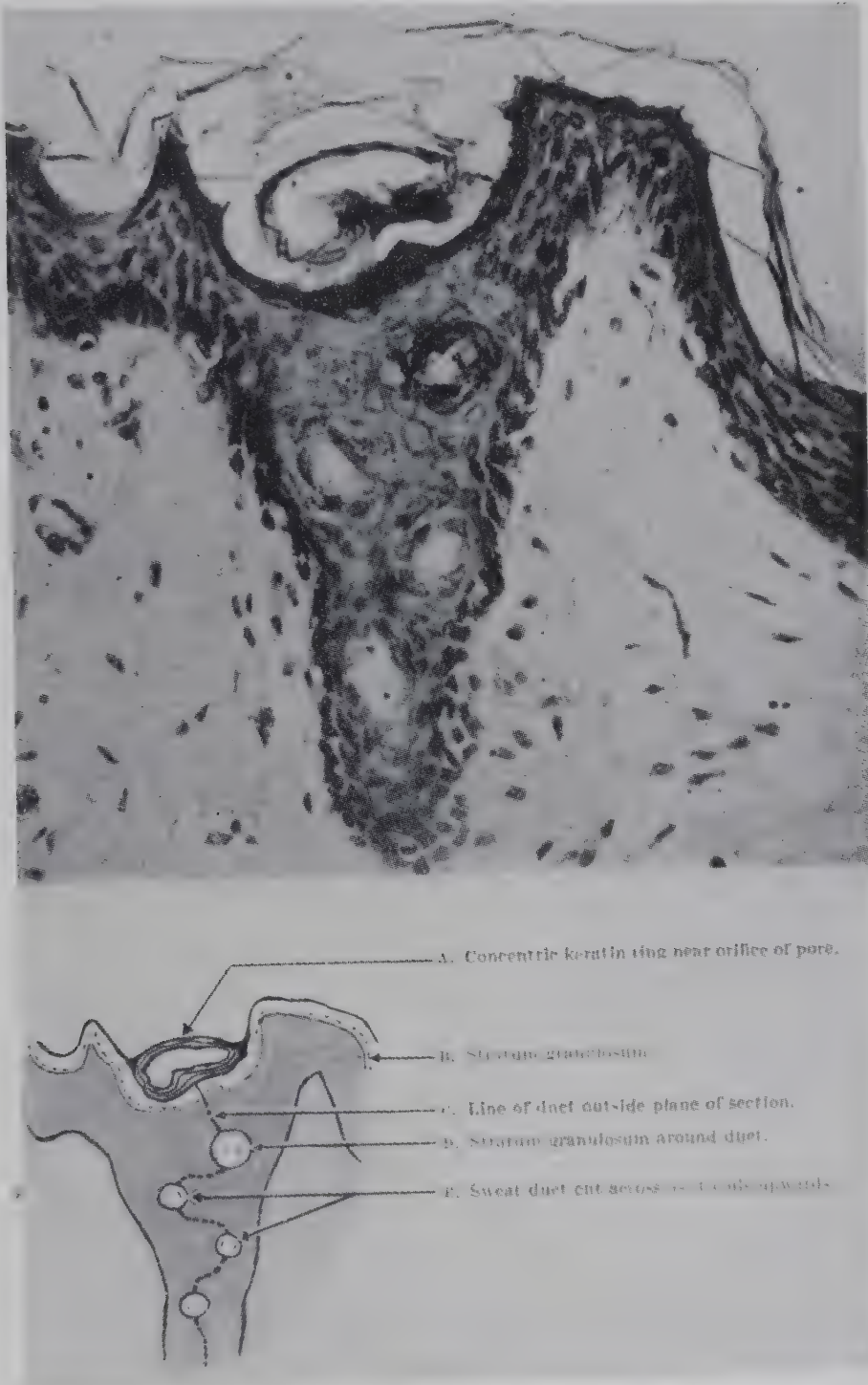


FIG. 1.—Terminal part of normal sweat duct with keratin ring. From O'Brien (10). (Reproduced by permission of the author and H. K. Lewis & Co., Ltd.) $\times 332$.

convolutions of the sweat ducts have walls composed of a laminated ringlike or tubelike condensation of keratin (Fig. 1). The closure of the pore occurs by occlusion of the lumen of this ring or tube by further accumulation of horny material (Fig. 2). The prime and essential change is this pathological hyperkeratinization which closes the lumen. In the next step, due to pressure by sweat that cannot escape, the duct dilates (Figs. 2 and 3), and its wall ruptures.¹ Sweat escapes into the neighboring tissue from the retention vesicle (Fig. 4). In a third phase a more voluminous keratin plug forms at the site of the vesicle. Below this plug the duct again dilates and ruptures at the epidermal-dermal junction or below it. This second rupture causes deep vesiculation, which corresponds to the "miliaria profunda" of Shelley (18, 15, 16, 17, 14) (Fig. 5). The secretory part of the sweat glands, though undergoing some dilatation, survives and obviously functions normally. In O'Brien's words, the sweat glands produce sweat and get rid of their secretion, no matter where they have to push it (13).

Sulzberger and his group first demonstrated that complete anidrosis from plugging of the sweat pores may occur without any grossly visible changes (25); that, after an extremely variable latency period, all

1. The rupture of the cuticle of the duct, as O'Brien describes it, was not seen by Weidman (29). Weidman wonders whether the microscopic vesiculation around the sweat duct is really due to seepage of sweat or whether the vesicle contains serum. Of course, minute amounts of sweat seeping through into the epidermis will cause irritation, with subsequent early vascular reaction and exudation of plasma. Even if plasma proteins should be demonstrated in these vesicles, this would not rule out the possibility that the lesion was initiated by sweat leakage.

classical clinical and histological signs of prickly heat may develop (20); and that, after the prickly heat rash has subsided, plugging and anidrosis may persist for several weeks (25). In conformity with O'Brien, Sulzberger *et al.* histologically demonstrated horny plugs in the sweat-duct orifices, edema around the ducts in the epidermis and upper corium, periductal cellular infiltration, and essentially unaltered secretory acini.

Sulzberger, Herrmann, and Zak (23) reported on sweat retention in patches of atopic dermatitis and seborrheic dermatitis. They brought forward convincing evidence that sweat retention can be a continuous complicating factor in these inflammatory skin diseases. The clinical significance of these findings is obvious. They represent the first rational interpretation of the striking effects of change of environment in atopic dermatitis. Also, by stating that sun-tanning produces a distinct and rapid beneficial effect on sweat retention, Sulzberger (20) supplied the first rational partial explanation of the beneficial effect of ultraviolet light irradiations in these diseases. Sweat retention from plugging of orifices was also demonstrated in contact dermatitis (8, 15), in ichthyosis, and in all kinds of healing inflammatory processes (5). Another mechanism of sweat retention, based on destruction of the ducts, was studied in atabrine dermatitis (9). In addition to the direct mechanically and chemically irritating effect of retained sweat, it was considered that materials carried by the sweat may act as antigens causing sensitization when, instead of being poured onto the skin surface, they seep into living epidermal cells (23).

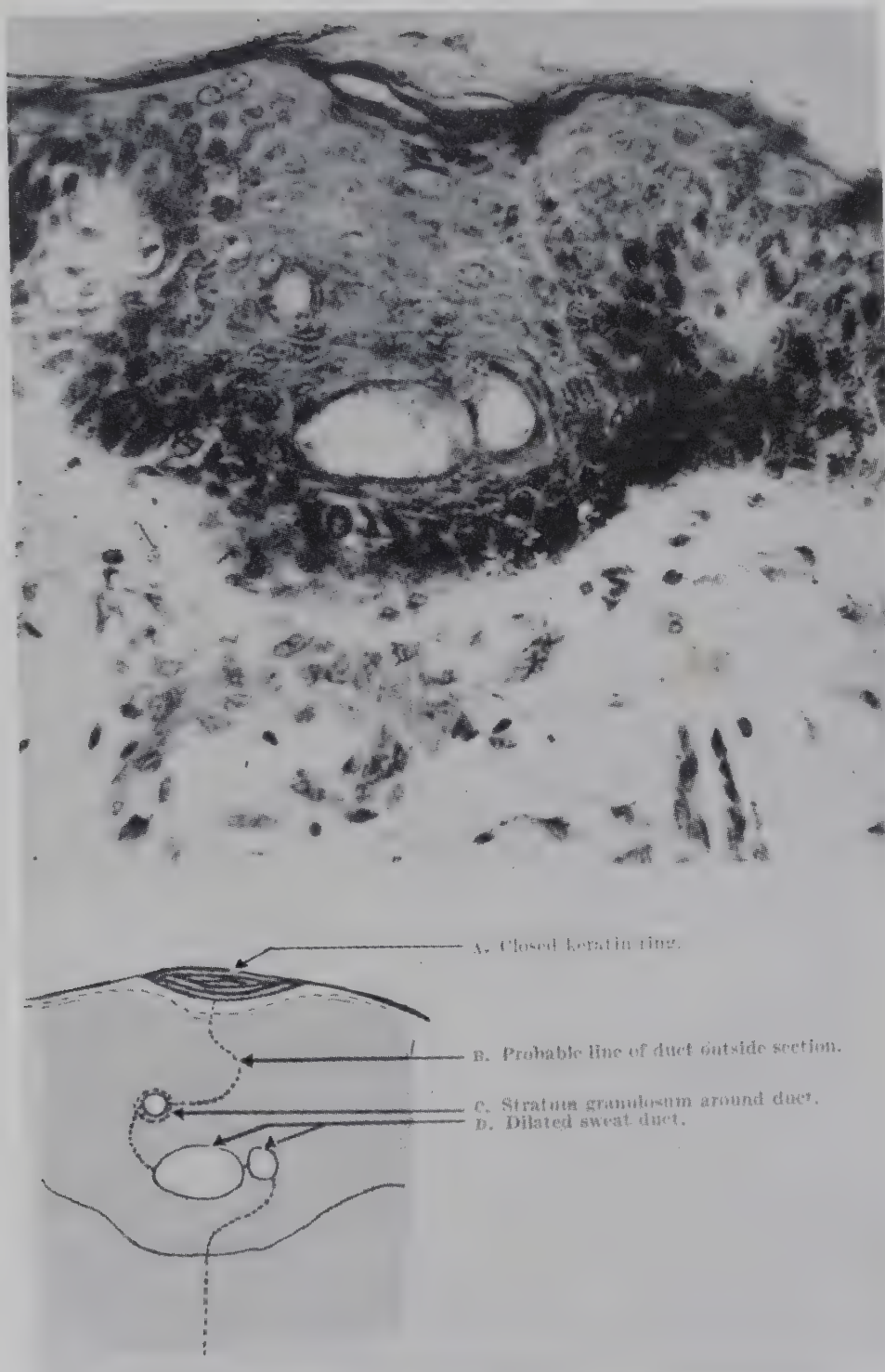


FIG. 2.—Closure of the keratin ring and dilatation of the deeper parts of the sweat duct. From O'Brien (10). (Reproduced by permission of the author and H. K. Lewis & Co., Ltd.) $\times 352$.

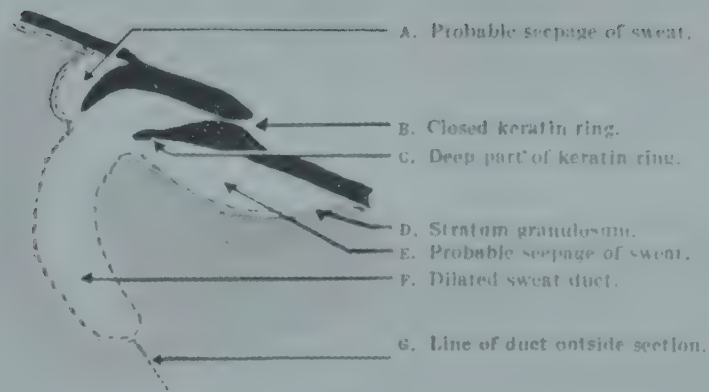
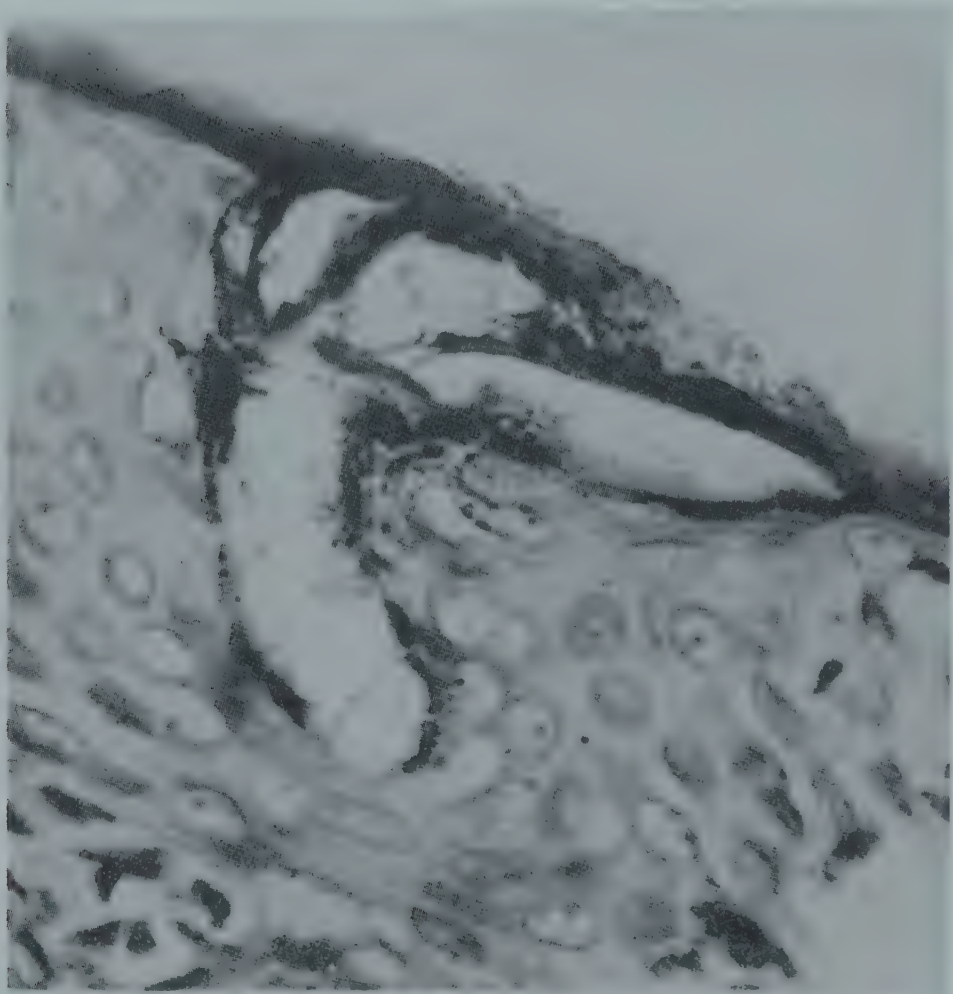


FIG. 3. —Closed sweat pore under high magnification. From O'Brien (10). (Reproduced by permission of the author and H. K. Lewis & Co., Ltd.) $\times 770$.

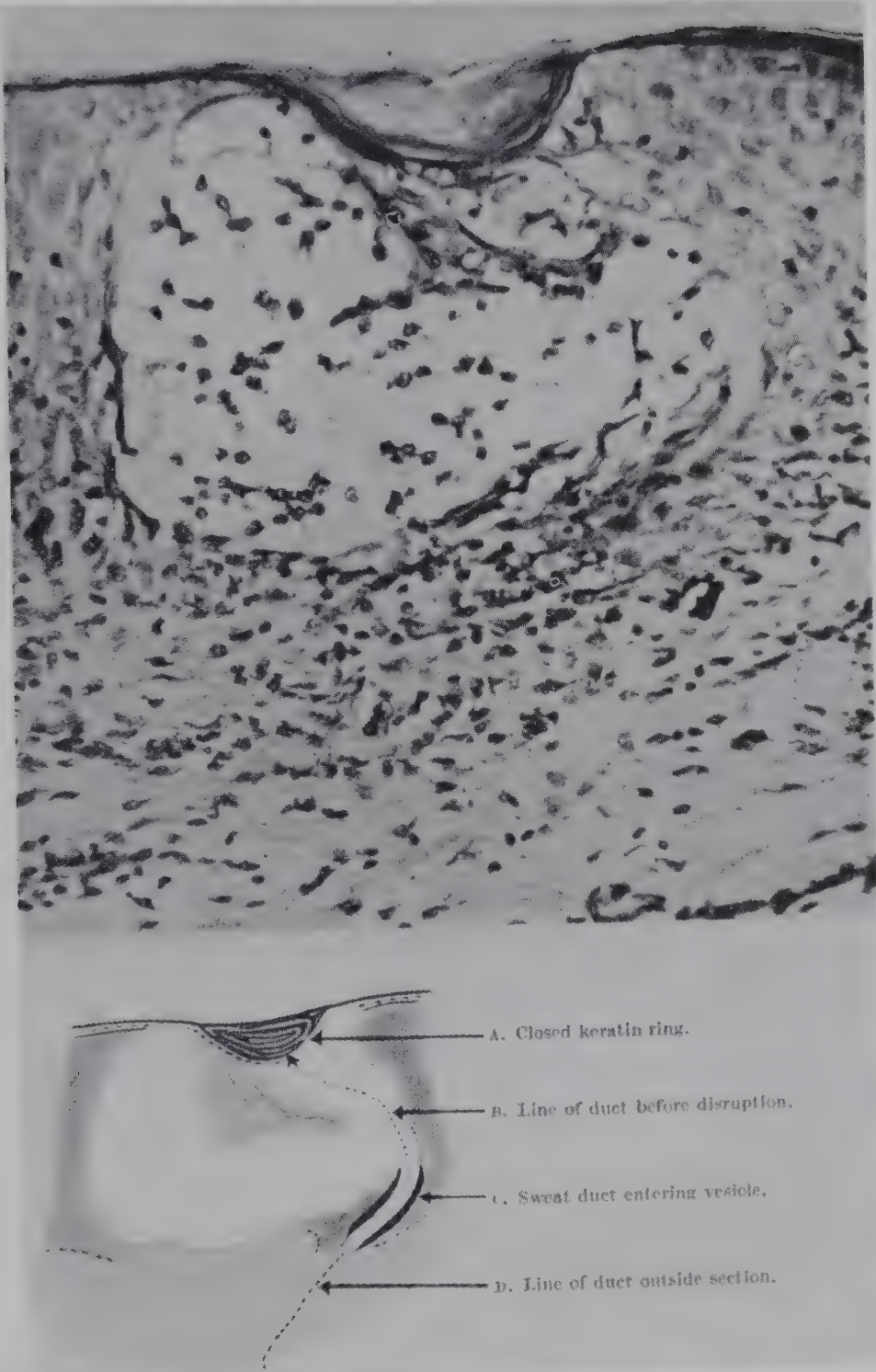


FIG. 4.—Miliaria vesicle. From O'Brien (10). (Reproduced by permission of the author and H. K. Lewis & Co., Ltd.) $\times 273$.



FIG. 5.—“Miliaria profunda.” Terminal sweat duct was destroyed 2 weeks before biopsy by diathermy current. Subject had been sweating for 1 hour before biopsy was taken. During this period of sweating, papules developed in the treated area. Note subepidermal vesicle, which reflects accumulation of sweat. Deep keratotic plug prevents retention of sweat in the epidermis. From Shelley (14). (Reproduced by permission of the Williams & Wilkins Company.)

II. THE EFFECT OF INJURY

Extensive experimental work in this field was done by Shelley and his associates (18, 15, 16, 17, 14) who studied the sweat-retention syndrome under strictly controlled conditions. The main result of Shelley's work has been the finding that plugging of the orifices can be produced not only by hydration ("maceration") of the horny layer, as it occurs in a hot, humid atmosphere or under wet dressings and under adhesive tape, but also by a great variety of minor and major epidermal injuries, such as galvanic current, heat, cold, ultraviolet light, and irritating chemicals, such as aluminum chloride, phenol, and chloroform (15). Thereby it was shown that hydration of the horny layer is not a necessarily decisive factor in sweat retention; that the decisive factor is an abnormal poral keratinization with hyper- or parakeratotic plug formation which is elicited by any kind of nonspecific epidermal injury. That the basic change is a keratinization anomaly is borne out by the fact that measures producing desquamation rapidly restore the patency of the pores (20, 15). The pore-keratinization response shows great individual variations, and these variations may account for differences in susceptibility to various exogenous and endogenous skin injuries.

Shelley (14), by grading injuries, produced experimentally the different clinical manifestations of sweat retention, viz., miliaria crystallina (formation of crystal-clear tiny vesicles on top of the orifices) (Fig. 6), presumably containing pure sweat (18, 15); miliaria rubra (sweat-retention vesicles with clinically visible signs of inflammation) (Fig. 7); and miliaria profunda (formation of papules with deep-seated fluid accumulation) (Fig. 8). The type of change reflects the level at which retention of sweat occurs. The most superficial site for the collection of sweat is within the stratum corneum, and such retention appears clinically as miliaria crystallina. At a lower level but still within the epidermal layer, accumulation of

trapped sweat results in miliaria rubra. Finally, miliaria profunda, produced experimentally by localized galvanic destruction of the straight part of the duct, comes about by a deep plugging of the duct. With sweat retained at the level of the horny layer, no inflammatory reaction results, because living cells are not damaged by tension or by seeping of sweat into the neighboring parts of the horny layer. When sweat is retained in the living part of the epidermis, seeping of sweat into the rete causes inflammatory reaction. When the horny plug is long and the accumulation of sweat occurs in the corium, the inflammation is again mild, if there is any, because sweat is more readily absorbed from the interfibrillary spaces of the corium and living cells are not invaded.

Concerning the effect of ultraviolet light on the patency of sweat pores, there are some conflicting results. These can be reconciled in a fashion somewhat similar to the one Shelley suggested (16): irritating (erythema-producing) doses cause epidermal injury promoting plug formation, whereas the subsequent desquamative period aids in the removal of the plugs. Continuous chronic exposure to ultraviolet light certainly aids in keeping the pores open (20). Simons (19) found that in the tropics Europeans are much more likely to suffer from prickly heat than natives are. This observation may be linked with the finding that in dark-skinned people the thickening of the horny layer during initial exposures to ultraviolet light is not regularly demonstrable (7). The marked plugging action of erythema doses of ultraviolet light was demonstrated by Thomson: the sweating rate decreased on the second to third day after exposure by up to 60 per cent, and "sudamina" (miliaria crystallina) resulted (27, 28).

All clinical manifestations of sweat retention occur only if the areas with plugged sweat pores are subjected to sweat stimuli. Shelley was able to prevent completely the development of lesions in his artificially



FIG. 6.—Sweat-retention vesicles. Postiontophoretic area of anidrosis. Subject had been treated 5 days previously with an iontophoretic current density of 1 M.A. cm^2 for 10 minutes, using distilled water under the anode. Prior to photography and spraying with quinizarin, the subject had been in the heat cabinet for 30 minutes. Dark spots are freckles. From Shelley *et al.* (18). (Reproduced by permission of the Williams & Wilkins Company.)

produced anidrotic areas by atropinization and to produce them by applying sweat stimuli. He stated (16) that there are three separate factors in the development of miliaria: (1) nonspecific epidermal injury, causing plugging; (2) intensive sweating for several hours; and (3) individual susceptibility. Also it was pointed out that many cutaneous

by W. C. Lobitz, Jr. (5). It occurs as a complication in the healing of skin diseases. It is not an infection of the skin, even though the lesions are similar to infectious periporitis. The lesions disappear spontaneously if sweating is prevented.

O'Brien (10) has observed that pore-plugging can be released by application of

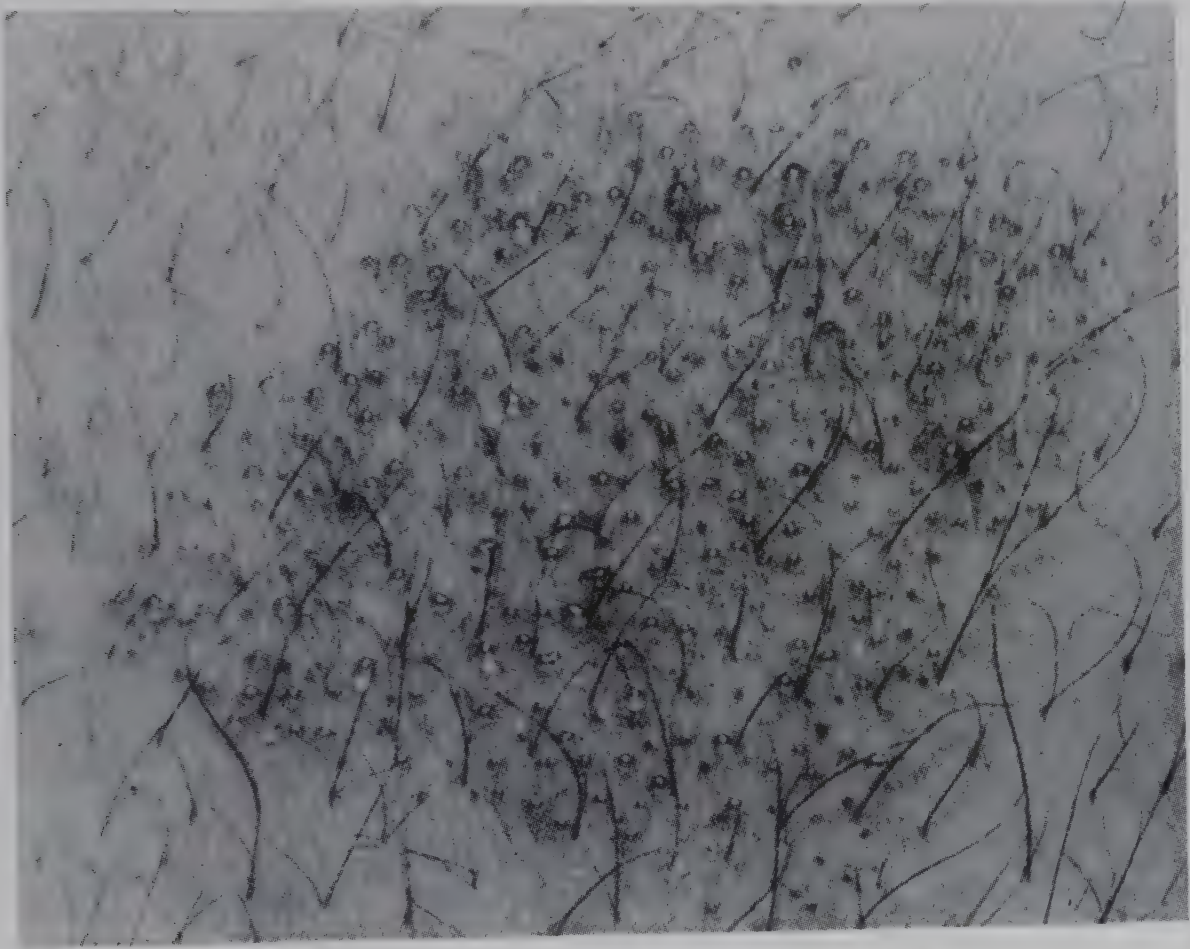


FIG. 7.—Experimental miliaria. Subject had been treated 5 days previously with iontophoresis, using distilled water and a current at the anode of 2.5 MA/cm² for 2 minutes. Picture taken after 3 hours of sweating in the heat (*D.B.*, 96° F.; *R.H.*, 90 per cent) shows superficial clear vesicles with associated erythematous areolae. This type of miliaria is less common clinically than is the type consisting of discrete to confluent erythematous macules associated with small papules. From Shelley and Horvath (16). (Reproduced by permission of the Williams & Wilkins Company.)

reactions which were thought to be due to primary contact irritation, such as adhesive-tape reactions, diaper rashes, and reactions to antiperspirants, are, in most cases, manifestations of the sweat-retention syndrome (16).

A special form of sweat retention—pustular miliaria—was described and analyzed

anhydrous lanolin and to a lesser degree also by other, less hydrophilic, fatty substances. Originally, this phenomenon led O'Brien to believe that plugging might be caused by lipid depletion of the horny layer and that lipids have a vitally important role in normal keratinization. However, Shelley *et al.* (15, 16) found that the action of lipid sol-

vents in promoting the plugging phenomenon was dependent on their capacity for causing epidermal injury. Chloroform, being most irritating, caused plugging; but benzene, ether, xylol, and carbon tetrachloride had no effect. Thus it appeared that the injury caused by the solvents rather than the

lipid depletion of the stratum corneum is only an auxiliary factor in poral closure. Sulzberger *et al.* (21) believe that the waxy surface film of the skin helps to keep the pores open by its hydrophilic properties but that lipid depletion is probably only one of many factors promoting closure.

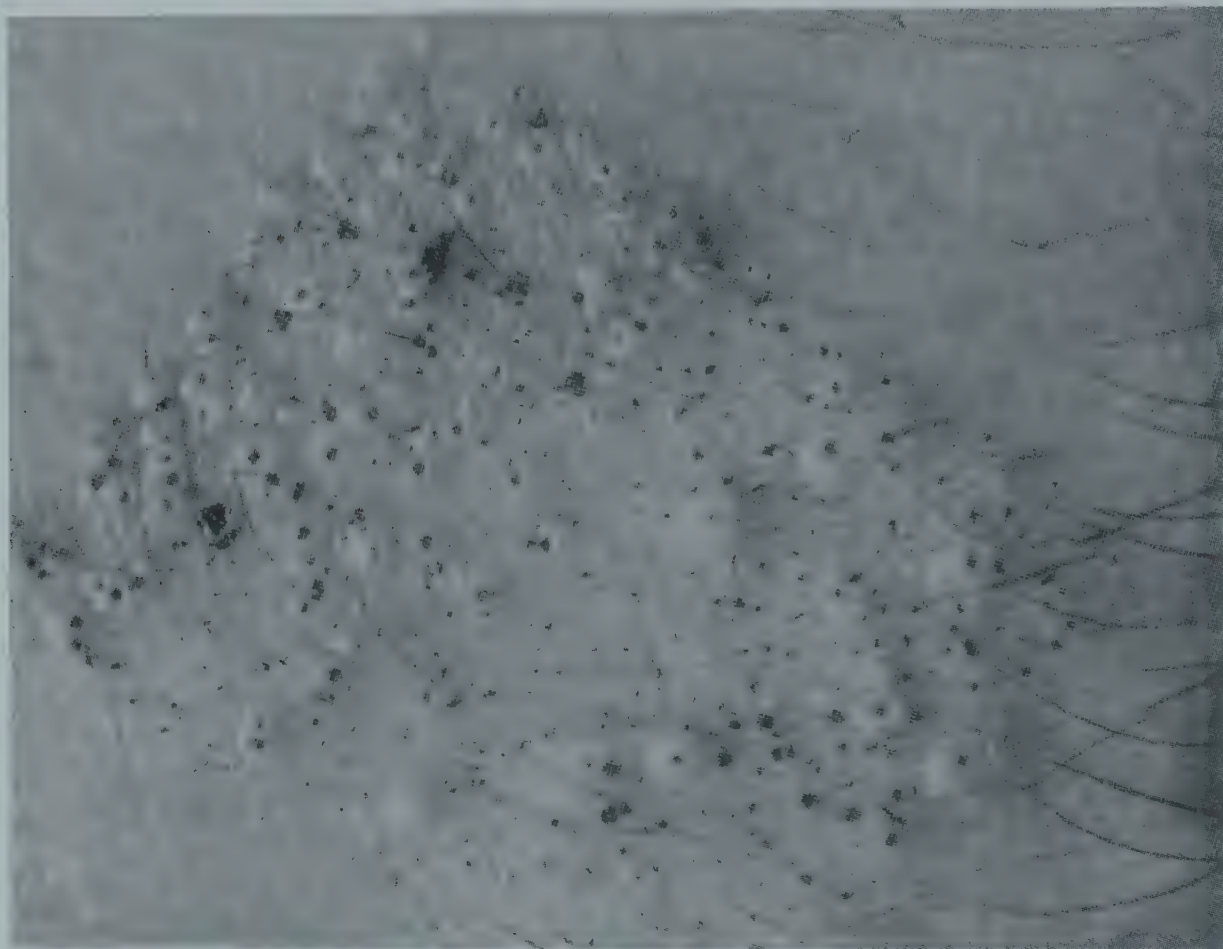


FIG. 8.—Sweat-retention “papules.” Rectangular site of induced sweat retention on forearm of subject who had received electrical injury to sweat ducts 24 hours before sweating was induced. Following 1 hour of sweating, these sweat-retention “papules” appeared. Note small dark puncta, which are the methylene-blue-stained sweat-gland orifices. The treated area reflects results of using two different settings on the Bovie electrosurgical unit. *Upper half*: contact with the needle, setting at 5 units, epilating current; *lower half*: insertion of needle, setting at 30 units, epilating current. This clinical appearance is to be compared with biopsy findings in Fig. 5. From Shelley (14). (Reproduced by permission of the Williams & Wilkins Company.)

removal of lipids was etiologically responsible for plugging. Shelley (15) was able to counteract the effect of chloroform by the addition of lanolin; but again the decreased plugging action paralleled a decreased irritating action (15). Recently, O'Brien (12) obtained similar results and concluded that

In spite of so many points having been clarified in the field of sweat retention, the basic problem—the mechanism of how and why a horny plug occludes the pore—has remained unsolved. The original assumption was that in a hot, humid atmosphere the horny layer hydrates, its volume increases,

and the poral closure results from this swelling (25). Shelley's experiments demonstrated that such swelling is not necessary for the development of poral closure. Furthermore, all authors agree that an active hyperkeratinization process on the pore and below is involved; it is not a swollen lamella of the horny layer which occludes the pore but a newly formed horny plug.

On the basis of all known facts, I believe that the most probable assumption is this: hyperkeratinization is the most common and most general reaction to any kind of moderate epidermal injury (p. 378). It appears that the keratinizing epithelium of the sweat-duct opening reacts more promptly to some epidermal injuries than does the keratinizing epithelium of follicular openings or that of the epidermis. Imbibition of sweat in hot, humid atmospheres seems to promote primary hyperkeratinization of the pore epithelium, because these cells are irritated the most. Probably, however, this is only a quantitative difference. Ultraviolet light,

for instance, causes not only poral closure but also hyperkeratosis of the epidermis (6, 7). Under other circumstances—for instance, in vitamin A deficiency—the keratinizing cells of the follicular pore react first; but again there is an additional hyperkeratotic reaction of the epidermis as a whole (p. 382). Thus poral closure becomes a special case of “superkeratinization reactions” (p. 378).

In this interpretation it becomes unnecessary to search for a “specific etiologic factor” of poral closure. O'Brien (12) has championed the idea that staphylococcal infection of the sweat pores is an important causative agent in the development of poral closure and miliaria rubra and has brought forward supporting evidence for this view. However, the comments on this work by Sulzberger and Herrmann (21), as well as the work of Shelley (18, 15, 16, 17, 14), clearly indicate that if infection ever plays a primary role, it represents only a special case in the mechanism of poral closure.

III. REPERCUSSIONS ON THERMOREGULATION

O'Brien (11) dealt in detail with the influence of sweat retention on heat regulation. He, like others, found only moderate hyperthermia, in spite of widespread anidrosis over trunk and limbs. The reason the body temperature does not rise to high fever levels is probably because the head and neck always perspire freely. Possibly there is even a compensatory hyperidrosis of these parts. Still, there is sufficient disturbance of the evaporative heat loss to cause a temperature elevation of 2° F. by exercise. Exercise also leads to considerable hyperpnea. O'Brien (11) believes that this “panting” is not a compensatory effort to increase evaporative heat loss through the respiratory passages. He was unable to demonstrate pronounced alkalosis with subsequent clinical tetany. Tetanic signs, however, were recorded by Ladell (4), and a tendency to alkalosis during exercise was demonstrated by O'Brien himself. Sulzberger (25) found that panting in this state does lead to considerable alka-

losis and that the disease symptoms are due to the shift in acid-base balance. O'Brien (11) emphasizes that this has never been clearly proved by blood analyses. In any case, it is probable that panting occurs because raised blood temperature and accumulation of carbon dioxide stimulate the respiratory center. Ladell (4) found that in some of his patients there was a possible functional element in the hyperventilation.

There is no dehydration and no true deficiency of sodium chloride in the sweat-retention syndrome. Ladell (4) found an unusually high sodium chloride concentration, i.e., 0.53 per cent, in the sweat of his patients; and his interpretation was that the glands, because of fatigue, were unable to maintain the usual great osmotic difference between blood and sweat. O'Brien postulated that the high salt concentration is due to compensatory hyperactivity of the healthy unobstructed glands. O'Brien denies any fatigue of the glands on the basis that

cessation of sweating is not preceded by profuse general hyperhidrosis.

O'Brien (11) relates the disease symptoms to a temporary peripheral circulatory failure: the effective blood volume is diminished by excessive dilatation of the peripheral vessels and cannot meet the needs of muscles and other internal organs during exercise. He is also inclined to believe that retained and reabsorbed sweat exerts some toxic influence.

O'Brien (11) proposed that if there is decreasing sweating ability with the advance of the hot summer season, this might be caused by a progressive plugging of the sweat pores. However, such "acclimatization" during summer probably is an exceptional event, the opposite being the rule (see pp. 164 and 265). On the other hand, O'Brien emphasizes that in acute heat-exposure experi-

ments, there is no obstruction of pores and that diminution of sweating, as demonstrated by Gerking and Robinson (2), is a true fatigue phenomenon.

According to O'Brien (11), there are three main forms of heat exhaustion: (1) heat hyperpyrexia or heat stroke, in which there is an intrinsic failure or paralysis of the thermoregulatory centers because of overstimulation; (2) the classic heat-exhaustion type I of Ladell, in which the essential cause of the disturbance is loss of water and salt; and (3) the sweat-retention syndrome, or type II exhaustion of Ladell. The analysis by O'Brien of cases observed in dry desert climates leads him to the conclusion that in such climates a combination of these forms is the rule, whereas in hot, humid climates the pure sweat-retention syndrome is prevalent.

BIBLIOGRAPHY

1. ALLEN, S. D., and O'BRIEN, J. P. Tropical anidrotic asthenia; preliminary report, *M. J. Australia*, **2**:335-36, 1944.
2. GERKING, S. D., and ROBINSON, S. Decline in rates of sweating of men working in severe heat, *Am. J. Physiol.*, **147**:370-78, 1946.
3. HERRMANN, F.; PROSE, P. H.; and SULZBERGER, M. B. Studies on sweating. IV. A new quantitative method of assaying sweat-delivery to circumscribed areas of the skin surface, *J. Invest. Dermat.*, **17**:241-49, 1951.
4. LADELL, W. S. S.; WATERLOW, J. C.; and HUDSON, M. F. Desert climate; physiological and clinical observations, *Lancet*, **2**:491 and 527, 1944.
5. LOBITZ, W. C., JR. Pustular miliaria, *J.A.M.A.*, **148**:1097-1100, 1952.
6. MIESCHER, G. Das Problem der Lichtstrahlen und der Lichtgewöhnung, *Strahlentherapie*, **35**:403-43, 1930.
7. ———. Die Schutzfunktionen der Haut gegenüber Lichtstrahlen, *ibid.*, **39**:601-18, 1931.
8. MILLER, C. S. Contact eczematous dermatitis and patch tests; histologic study, *Arch. Dermat. & Syph.*, **56**:678-94, 1947.
9. MILLER, O. B.; HERRMANN, F.; and RUBIN, J. The effects of mepacrine hydrochloride (atabrine) upon the human skin, *J. Invest. Dermat.*, **15**:445-52, 1950.
10. O'BRIEN, J. P. A study of miliaria rubra, tropical anhidrosis and anidrotic asthenia, *Brit. J. Dermat.*, **59**:125-58, 1947.
11. ———. Tropical anhidrotic asthenia; its definition and relationship to other heat disorders, *Arch. Int. Med.*, **81**:799-831, 1948.
12. ———. The etiology of poral closure; an experimental study of miliaria rubra, bul-
lous impetigo and related diseases of the skin. I. An historical review of the causation of miliaria, *J. Invest. Dermat.*, **15**:95-152, 1950.
13. ———. Lecture delivered at Billings Hospital, University of Chicago, March 31, 1952.
14. SHELLEY, W. B. Experimental miliaria in man. IV. Sweat retention vesicles following destruction of terminal sweat ducts, *J. Invest. Dermat.*, **16**:53-64, 1951.
15. SHELLEY, W. B., and HORVATH, P. N. Experimental miliaria in man. II. Production of sweat retention anidrosis and miliaria crystallina by various kinds of injury, *J. Invest. Dermat.*, **14**:9-20, 1950.
16. ———. Experimental miliaria in man. III. Production of miliaria rubra (prickly heat), *ibid.*, pp. 193-204.

17. SHELLEY, W. B.; HORVATH, P. N.; and PILLSBURY, D. M. Anidrosis, *Medicine*, **29**: 195-224, 1950.
18. SHELLEY, W. B.; HORVATH, P. N.; WEIDMAN, F. D.; and PILLSBURY, D. M. Experimental miliaria in man. I. Production of sweat retention anidrosis and vesicles by means of iontophoresis, *J. Invest. Dermat.*, **11**:275-91, 1948.
19. SIMONS, R. D. G. P. Studies on prickly heat, miliaria and sudamina, *Dermatologica*, **93**: 172-82, 1946.
20. SULZBERGER, M. B., and EMIK, L. O. Studies on prickly heat. I. Clinical and statistical findings, *J. Invest. Dermat.*, **7**:53-59, 1946.
21. SULZBERGER, M. B., and HERRMANN, F. Comments on "The etiology of poral closure, an experimental study of miliaria rubra, bullous impetigo and related diseases of the skin," by J. P. O'Brien, M.D., *J. Invest. Dermat.*, **15**:153-55, 1950.
22. SULZBERGER, M. B.; HERRMANN, F.; KELLER, R.; and PISHA, B. V. Studies on sweating. III. Experimental factors influencing the function of the sweat ducts; a preliminary report, *J. Invest. Dermat.*, **14**:91-109, 1950.
23. SULZBERGER, M. B.; HERRMANN, F.; and ZAK, F. G. Studies on sweating. I. Preliminary report with particular emphasis on a sweat retention syndrome, *J. Invest. Dermat.*, **9**:221-42, 1947.
24. SULZBERGER, M. B.; ZAK, F. G.; and HERRMANN, F. Studies on sweating. II. On the mechanism of action of local antiperspirants, *Arch. Dermat. & Syph.*, **60**:404-18, 1949.
25. SULZBERGER, M. B., and ZIMMERMAN, H. H. Studies on prickly heat. II. Experimental and histologic findings, *J. Invest. Dermat.*, **7**:61-68, 1946.
26. SULZBERGER, M. B.; ZIMMERMAN, H. M.; and EMMERSON, K., JR. Studies on prickly heat. III. Tropical anidrotic asthenia (thermogenic anidrosis) and its relationship to prickly heat, *J. Invest. Dermat.*, **7**:153-64, 1946.
27. THOMSON, M. L. Dyshidrosis produced by general and regional ultra-violet radiation in man, *J. Physiol.*, **112**:22-30, 1951.
28. ———. The causes of changes in sweating rate after ultra-violet radiation, *ibid.*, pp. 31-42.
29. WEIDMAN, F. D. Discussion on SHELLEY and HORVATH (16).
30. WOLKIN, J.; GOODMAN, J. I.; and KELLEY, W. E. Failure of the sweat mechanism in the desert; thermogenic anhidrosis, *J.A.M.A.*, **124**:478-82, 1944.

CHAPTER 12

Sebaceous-Gland Excretion

I. PHYLOGENETIC CONSIDERATIONS	284
II. STRUCTURE	285
III. MECHANISM OF EXCRETION	288
IV. REGIONAL DIFFERENCES	295
V. INFLUENCE OF AGE AND SEX	295
VI. ENDOCRINE INFLUENCES	297
VII. NUTRITIONAL INFLUENCES	302
VIII. THE PROBLEM OF INNERVATION	303

I. PHYLOGENETIC CONSIDERATIONS

FISHES and amphibians have no cutaneous glands which could be regarded as forerunners of sebaceous glands (5). However, the mucinous glands of some slimy fishes (Myxinidae) and the poison glands of fishes and amphibians have been compared with sebaceous glands as being primary examples of the development of holocrine ("polyp-tych") glands from the epidermis (69), i.e., they are multilayered glands, the "secretion" process of which involves total disintegration of their cells. From the functional point of view they remind one of sebaceous glands because their excretion products lubricate the surface and protect against the colonization of plant and animal parasites. Also some of these glands are under the influence of sex hormones; their volume increases considerably during periods of sexual activity (5). Physically and chemically, most of these excretion products behave like mucins, although they do not contain a carbohydrate group (5). They are highly viscous and can be precipitated by weak acids. Some of them, however, contain considerable amounts of lipid substances.

For instance, 25 per cent of the dry material of the cutaneous slime of the eel is made up of cholesterol and phosphatides (51). Yet, on the whole, all these glands are more similar to mucous glands than to sebaceous glands.

Some cutaneous glands of reptiles, however, are considered to be phylogenetic ancestors of mammalian sebaceous glands, such as the femoral glands of lizards, the cloacal glands of crocodiles, and the anal glands of turtles and giant serpents (69). All these organs are sacklike indentations of the epidermis, the cells of which undergo fatty decomposition before they are cast off.

In the mandibular and cloacal glands of alligators, keratinization and fatty decomposition coincide conspicuously (58). While the periphery of the cell keratinizes, its center undergoes fatty decomposition. In the end the cells disintegrate like sebaceous-gland cells. Thus they can be regarded as transitional forms between undifferentiated epidermal cells which are destined to keratinize and cells which are differentiated into gland cells, the final fate of which is fatty decomposition. If one remembers that, in

mammals, vernix caseosa and smegma are, in the main, not of glandular origin but are directly fatty decomposition products of epidermal cells, one may speculate that all epidermal cells have the potentiality of fatty decomposition. Indeed, the separation of lipid material as it occurs all over the skin surface of mammals during keratinization (see p. 374) is an indication of this potentiality.

The preen gland (*glandula uropygialis*)—the only cutaneous gland of birds—is a bilobular sebaceous gland. It is situated in the coccygeal region at the base of the tail. Its fatty product is taken up by the beak and smeared over the feathers. It is best developed in water birds, in which the oiling of feathers is most needed for protection against the macerating effect of water. Hou (31) found that after extirpation of the preen gland in ducks and other birds the plumage became discolored, looked dull and soiled, and lost its smooth contours. Also there was increased shedding of feathers. Oil droplets, normally present in great numbers between the barbs, barbules, and barbicels, disappeared after the operation. If the birds were forced to swim in cold water, there was a greater fall and slower recovery in body temperature than in normal controls. Thus it was proved that sebaceous-gland function is essential for protection against heat loss in these animals.

In addition, Hou (32, 33) found that operated birds suffered a progressive impairment of general health and eventually died,

displaying signs suggestive of rickets. It could be shown that these signs actually were caused by vitamin D deficiency. Physiologically, vitamin D is formed in the plumage of normal birds by the ultraviolet radiation of the vitamin precursor, which originates in the preen glands and therefore is lacking in the plumage of operated birds. That the precursor is present in preen oil was shown by feeding irradiated preen oil to operated birds and thereby preventing rickets. Dietary rickets could be cured by ultraviolet irradiation of birds with intact preen glands but not if the preen gland was removed.

An accidental observation of Hou (32) threw light on the failure of previous workers to demonstrate the physiological significance of the preen gland in water birds. A French author, who had negative results with operated animals, kept operated and control birds in common cages. When Hou did the same thing, he noticed that the operated animals instinctively tried to “steal” some sebum from their fellow-birds by picking at their glandular orifices with their beaks. The French author himself noticed that the control birds behaved in a “hostile manner” toward the operated ones but could not explain this behavior.

In mammals, according to Schaffer (69), the sebaceous glands, like the apocrine coil glands, excrete species- and sex-specific odorous substances which play a role in sexual attraction and help in the recognition of the same and foreign species.

II. STRUCTURE

In man most sebaceous glands develop from the follicular epithelium of the hair (Fig. 1). The earliest anlage consists of a spherical bud composed of cells of the external root sheath. This bud develops into a multilobulated gland surrounding one-half to two-thirds of the circumference of the hair follicle (Fig. 2). A short duct connects the lobules with the follicular canal.

On microscopic cross-sections, a basement membrane is seen surrounding the lobules.

The outermost cell layer or basal layer consists of flat epithelial cells. In the second layer inward, the cells are larger and show large fat droplets within their cytoplasm (Fig. 3).¹ In subsequent generations the accumulation of fat progresses, and the cyto-

1. The formation of fat droplets was also observed in the basal cells. If in this way the whole lobule disintegrates, regeneration occurs from the epithelium of the duct or from the external root sheath (49).

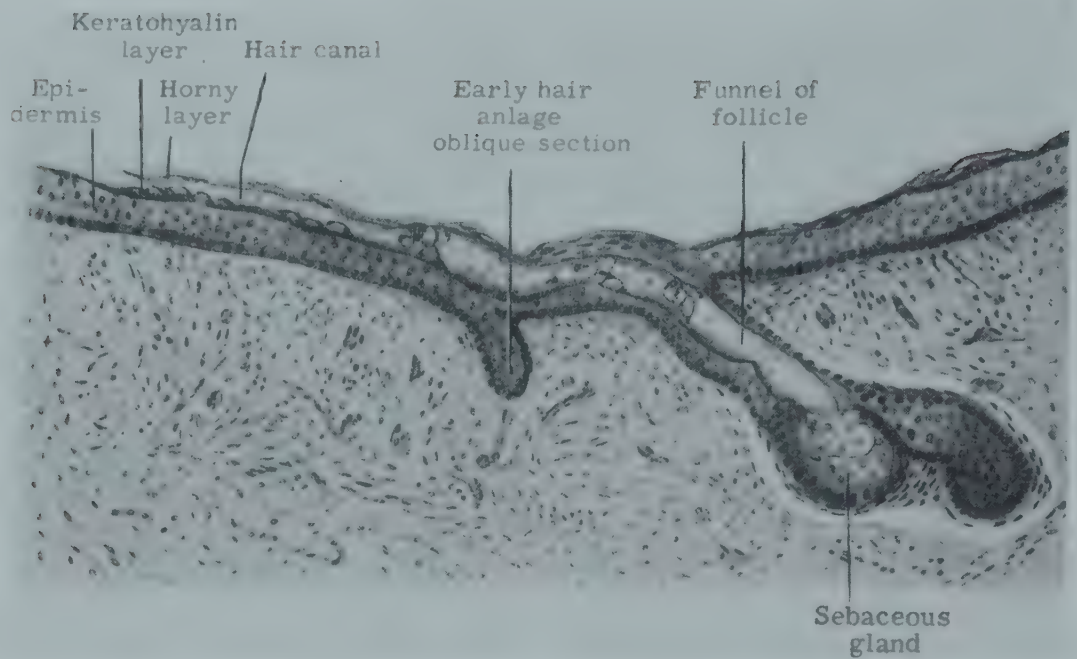


FIG. 1.—Development of the sebaceous gland from the follicular epithelium of the hair. From Pinkus (59). (Reproduced by permission of Springer-Verlag.)



FIG. 2.—Plastic reconstruction of a sebaceous gland. From Pinkus (59). (Reproduced by permission of Springer-Verlag.)

plasm becomes reduced to a reticular meshwork. As contrasted with fat formation in the subcutaneous tissue, the fat droplets remain separated from one another through several cell generations. Finally, the cell membrane and the protoplasmic meshwork break down, and an amorphous fatty mass results, which diffuses into the follicular canal. From there it diffuses upward, im-

fat formation in sebaceous glands is a physiological function, while the term "degeneration" implies a pathological event. Still it can hardly be denied that the physiological disintegration of sebaceous-gland cells closely simulates pathological fatty degeneration. Certainly, for the designation of the process involved, the word "excretion" is better than "secretion."

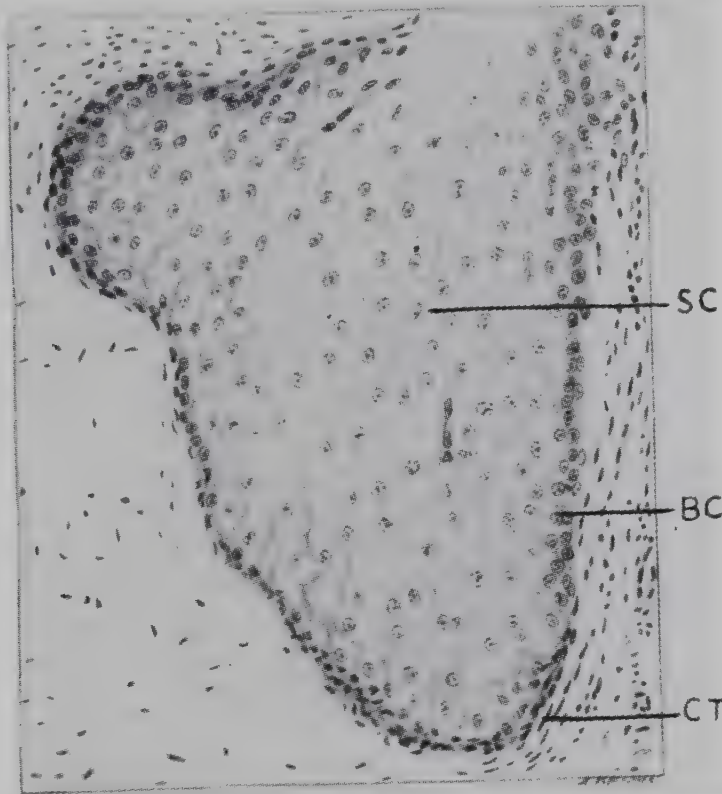


FIG. 3.—Section through a normal sebaceous gland. SC are sebaceous cells; BC are basal cells; CT are connective-tissue cells as a pseudo-membrane upon which the basal cells rest. From Lee McCarthy, *Histopathology of the skin* (St. Louis: C. V. Mosby Co., 1931). (Reproduced by permission of Dr. McCarthy.)

pregnates the hair and the horny layer, and takes part in forming the greasy film of the surface. What is seen under the microscope corresponds to an unparalleled biochemical process of an extremely rapid transformation of the basal cell protoplasm into a mixture of lipids with an amazing multitude of highly specific compounds (chap. 13). In the past there has been much discussion as to whether the fate of sebaceous-gland cells can be designated as fatty degeneration (or as the "fatty metamorphosis" of Virchow). The objection to such terminology was that

In man sebaceous glands occur everywhere where hair follicles are present, i.e., all over the skin surface except soles, plantar surfaces and interspaces of toes, side surfaces of feet halfway up to the ankles, palms, palmar surfaces of fingers, and the palmar half of the interspaces of fingers. The size and shape of sebaceous glands differ considerably in different regions. They are largest in the skin of the forehead, face, neck, and upper chest and are relatively small on the remaining parts of the trunk and on the extremities. On the hairy scalp they are

fairly well developed but form rather elongated alveoli, as contrasted with their rounded shape elsewhere. The number and size of sebaceous glands in different regions at different ages were studied by Okajima and his associates in great detail (55).

In addition to sebaceous glands which are attached to hair follicles, there are so-called "free" sebaceous glands which occur independently of hair follicles. They can be found mainly in regions transitional between the skin and mucous membranes. Some of these free glands are present in every person, such as the Meibomian glands of the eyelids. Frequently, but not in every person, sebaceous glands occur on the vermillion border of the upper lip, buccal mucous membranes (in linear shape across the dental closure, so-called Fordyce "disease"), prepuce, glans, labia minora, anus (at the transition from the cutaneous to the columnar part), nipple, and areola. Some of these glands open with a patent duct to the surface; others are inclosed under the surface, and, if they produce sebum at all, it never reaches the surface but becomes absorbed. They can be single or multiple, and in the latter case they are reminiscent of sebaceous-gland organs of lower forms.

According to Schaffer (69), there are

primary and secondary free sebaceous glands, viz., (1) ontogenetically deriving directly from the epidermis or transitional mucous membranes and (2) becoming free because of secondary atrophy of the hair follicle (as is the case in early male baldness) or "free" because of excessive development of the sebaceous glands and rudimentary development of hair follicle from the beginning.

The external ear canal has two kinds of cutaneous glands—true sebaceous glands and large alveolar apocrine "ceruminous" glands (50). Thus cerumen is a mixed product of these two glands.

Smegma, no matter whether sebaceous glands are present in the prepuce or not, is not a glandular product but is formed by fatty metamorphosis of the epithelial cells of the surface.

While some authors regard the hair follicle as the phylogenetically older system (59), others (69) insist that sebaceous glands are the primordial cutaneous appendages.

Changes in the proliferation of sebaceous-gland cells are correlated to the hair cycle (56). Parnell (56) found cyclic changes also in the dermis and considered all these changes as components of an aggregate skin cycle (p. 612).

III. MECHANISM OF EXCRETION

The most conspicuous and probably unique feature of sebaceous-gland excretion in man is that if one starts out with a thoroughly defatted surface, fat excretion sets in at a rapid rate but that this rate gradually declines, and further expulsion of fat finally stops or falls to a minimum rate when the fat layer has reached a certain thickness. The rapid excretion is resumed when the fat layer is removed again.

This phenomenon was first observed by Schur and Goldfarb (71) in 1927. Soon after their publication the significance of these findings was emphasized, and I pointed out that in collecting data it would be necessary in the future to differentiate the rate of excretion, as measured in short time intervals

after defatting, from the measurement of the saturation level, which represents the maximum amount of fat that can accumulate on the surface (68). Nevertheless, for more than eight years not much attention was paid to this phenomenon, and no further work was done on it. In 1936 and 1938 Emanuel (19, 20) and Serrati (73) independently re-examined the phenomenon of decreasing excretion rate and standstill and found it essentially confirmed. But, while Schur and Goldfarb noted stoppage of excretion as early as 15 minutes after fat removal, the data of Emanuel and of Serrati indicated that the excretion ceases completely only after several hours. The time required for restoration of the original level was found to

be longer than 3 hours by Emanuel (20), 3–4 hours by Zehender and Dünner (85), and within 3 hours by Pritchard *et al.* (61). If one follows the excretion curve over different periods of time up to several weeks (20), starting each period with removal of the surface fat layer, one finds an abrupt rise in the amount of fat excreted for 2 or 3 hours. This, however, is followed by a flattening and leveling-off of the curve (Fig. 4).

When the fatty film is removed from the skin surface in subsequent short periods of time, the sum of these fat portions is always much larger than it is when, for an identical total period, the accumulating lipids are left untouched on the skin surface (48, 15, 28). This difference led Miescher and Schönberg (48) to distinguish two quantities—the “saturation level,” representing the maximum amount of lipids which accumulates when the layer is left alone, and the “production capacity,” the amount of lipids expelled to the surface per unit time and unit surface within the period when a leveling-off has not yet taken place. Recently, the latter quantity was called “replacement sum” (28). Probably the best term for it is “excretion rate.” Lately it has been expressed in micrograms per square centimeter per minute (38).

Herrmann and Prose (28) pointed out that two saturation levels can be distinguished: one which is obtained when the surface is completely protected from accidental removal of fat (total or true saturation level) and a “casual level,” which is obtained when the test site is not protected against accidental touching, wiping, or contact with clothing and bedding. The true saturation level is about twice as large as the casual level (for instance, an average of 3.38 mg. versus 1.77 mg. per square centimeter on the forehead in Herrmann and Prose’s experiments) (28). Information on the casual level is important because it reflects the situation under conditions of everyday life. Actually, a great number of investigators measured the casual level, even if they advised the experimental subjects to refrain from touching the experimental areas. On

the forehead, for instance, it is impossible to avoid contact with the bedding at night. At saturation levels, simple wipings do not remove all the surface fat. Such removal requires repeated washings (38). Correspondingly, the concept of a “retained level” was introduced, i.e., the level remaining after a standardized type of wiping (28).

In all these experiments the total amount of skin surface fats was measured, and it must be kept in mind that this fat has at least two different sources, viz., sebaceous glands and keratinizing cells (p. 374). However, the contribution of epidermal lipids is relatively small (28), and there is good reason to believe that the saturation pattern

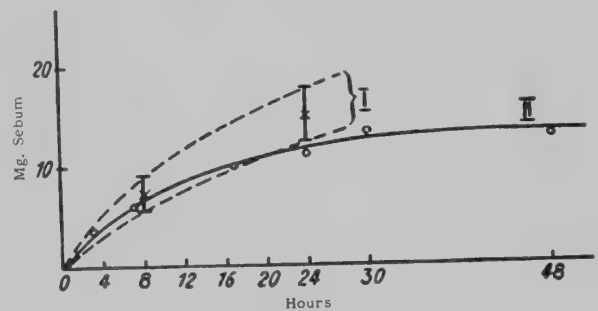


FIG. 4.—Range of saturation level in the same subject. From Dünner (15). (Reproduced by permission of S. Karger.)

just described is primarily a peculiarity of sebum excretion (p. 310).

Emanuel (19, 20) interpreted the saturation pattern as being the result of two opposite forces. One force is the “glandular pressure,” which is counteracted by a counterpressure represented by the viscous surface layer of the excreted sebum. The counterpressure will overcome the glandular pressure after a certain thickness of lipids has been deposited on the surface, and then further expulsion of sebum will stop. The thickness of the layer at which standstill occurs depends on the viscosity of the sebum. More viscous sebum stops further expulsion earlier than does a more fluid one, because more viscous sebum exerts greater counterpressure. After saturation has been reached, sebum will still accumulate in the duct but will be evacuated only if the surface layer is

removed. Evacuation from the duct gives the impulse to the glandular cells to resume their activities.

Emanuel (19, 20) contended that the glandular pressure of a single gland is proportional to the number of cells making up the glands, or, in other words, to the size of the gland. "Sebum is excreted alone through the force derived from the proliferation of the gland." This contention has received brilliant confirmation by the work of Mie-

The most remarkable feature in this work was that the quotient was about the same not only under physiological conditions but also in extreme cases of pathologically increased flow of sebum, namely, in juvenile and in postencephalitic seborrhea. These results indicated that if there is an increased flow of sebum, it is not caused by increased excitation and increased function of the glands, as it would be in any other gland of the body, but merely by an increase in glandular size. How much sebum a gland produces depends on the mitotic activity of its basal cells.² If this is greater than normal, more cells and more sebum are produced.

The excessive flow of sebum in Parkinsonism was previously interpreted as a direct consequence of brain damage in which an inhibitory nervous center of sebaceous-gland function had been destroyed so that the flow became uninhibited (p. 304). Miescher and Schönberg came to the conclusion that if there is any central nervous influence at all on sebaceous glands, it must be not an inhibition of excretion but an inhibition of pathological overgrowth of sebaceous glands. The skin in juvenile and in postencephalitic seborrhea differs from normal skin by an immense hyperplasia of its sebaceous glands, the degree of which directly accounts for the degree of increased flow.

It was noted a long time ago that sebaceous-gland function depends greatly upon the atmospheric temperature (68). Since the work of Emanuel, it has become clear that one important mechanism whereby temperature determines the final sebum level at which standstill occurs is the change in viscosity of the sebum. The "melting point" of sebum is around 30° C., which is close to the temperature of the skin surface (p. 258). At high temperatures sebum is liquid; at moderate temperatures, semiliquid; and

2. Both Dr. Bullough and Dr. Ebling believe, on the basis of recent evidence, that the mitotic activity and potentiality of cells to form sebum are independent phenomena and can be separated experimentally (personal communication; see also W. S. Bullough and F. J. Ebling, Cell replacement in the epidermis and sebaceous glands in the mouse, *J. Anat.*, 86: 29-34, 1952).

TABLE 1*

RELATIONS BETWEEN "PRODUCTION CAPACITY" (12 SUBSEQUENT 2-HOUR EXPERIMENTS), SEBUM LEVEL (24-HOUR EXPERIMENT), AND TOTAL GLANDULAR SURFACE (48)

	12× 2-Hr. Experiment (Amount of Sebum/ Glandular Surface)	
S. M., normal person.....	$\frac{17.8}{11.6}$	= 1.36
P. M., normal person.....	$\frac{18.9}{14.7}$	= 1.29
Schmid, seborrhea.....	$\frac{108}{77.1}$	= 1.4
Maurer, postencephalitic seborrhea	$\frac{32}{19.9}$	= 1.67
Vogel, postencephalitic seborrhea .	$\frac{84}{60.9}$	= 1.38
Wild, postencephalitic seborrhea ..	$\frac{100}{70}$	= 1.43

* Amount of sebum expressed in milligrams; surface area expressed in arbitrary units.

scher and Schönberg (48). These authors measured saturation levels and excretion rates. Subsequently, they biopsied the areas on which these estimations were made and measured planimetrically the area of glandular surface relative to the area of the whole microscopic section. They found that the secretion rate was directly proportional to the relative size of the glandular surface. The secretion rate (expressed in milligrams of fat) divided by the area taken in by sebaceous glands (expressed in arbitrary units) yielded a fairly constant quotient (see Table 1).

at low temperatures, as solid as a hard ointment. At low temperatures the sebum solidifies and counteracts further expulsion of sebum at a considerably lower level than at higher temperatures. Quantitatively, this phenomenon was studied first by Dünner (15). As shown in Figure 5, he found extremely low values at freezing temperatures and a progressive increase of excretion as the temperature was raised. For instance, comparable saturation values at 50° and -2° C.

position of the sebum. Individual variations in the shape of the saturation curve (Fig. 6) at a given temperature were interpreted as being due to differences in composition. Miescher (48) assumed that the melting point is low when fatty acids prevail in the lipid mixture and high when the mixture contains mainly cholesterol and wax alcohols. But this suggestion was not followed up. In pathological cases of seborrhea it seems that sebum is more liquid than nor-

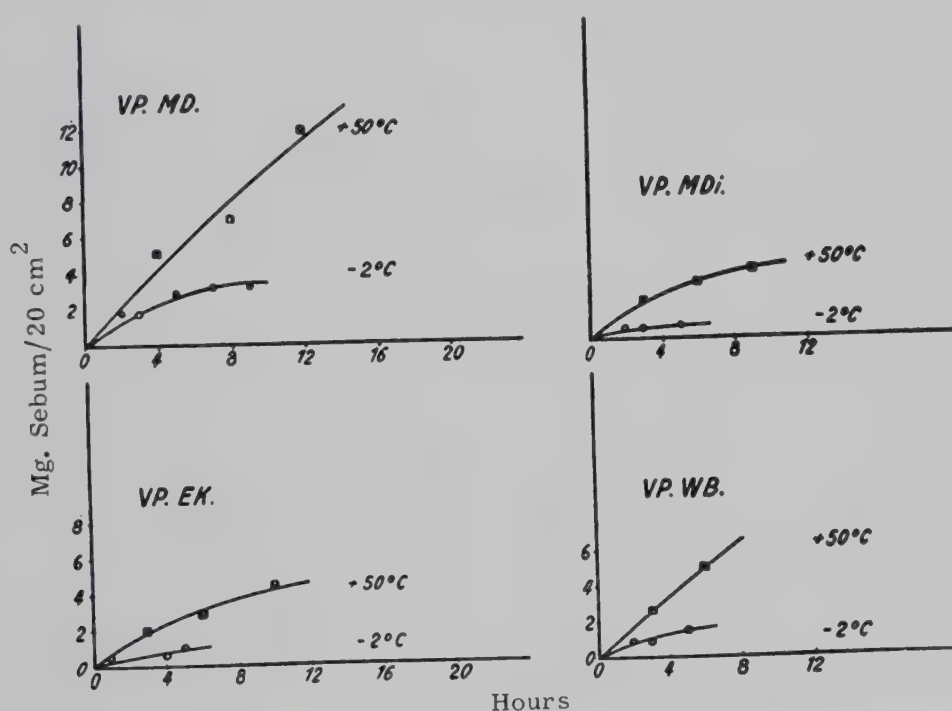


FIG. 5.—Effect of temperature on the saturation level in four subjects. 20 sq. cm. surface. From Dünner (15). (Reproduced by permission of S. Karger.)

were 11.8 and 3.1 mg. or 3.7 and 0.7 mg., respectively. The possible clinical significance of these findings for “dry skin” and particularly for pruritus hiemalis (“winter itch”) is discussed on page 378. At high temperatures the standstill of sebaceous excretion occurs rather late or not at all (Fig. 5). Sebum may become so fluid as not to form an occlusive layer at all. It just flows away, and the leveling effect may be entirely absent. An additional factor at high temperatures, recognized recently (28), is sweat secretion. Moisture emulsifies sebum and facilitates its spread (38).

The consistency also depends on the com-

mal. Whether this is caused by a difference in composition, by an abnormal amount of sweat mixed with the sebum, or by some other factor is not known. In postencephalitic seborrhea Serrati (73) conclusively demonstrated that there is an actual “flow” of sebum, with no tendency toward leveling. Thus the expression “seborrhea,” or flow of sebum, is indeed justified.

Once expulsion has ceased because of the occlusion of the pore by frozen sebum, production of sebum continues, at least for a while. This was shown by Dünner (15). He allowed sebum to accumulate to the saturation level. Subsequently, he removed the

layer and measured the excretion rate in 2-hour periods. The yield in the first period was considerably greater than in subsequent periods. Starting with the second period, the hourly rate became constant (Fig. 7). The larger yield of the first-hour sample was attributed to excess accumulation of sebum in the follicular canal at a time when expulsion was not possible. Accordingly, Dünner says,

it seems that it is not the "glandular" pressure which is overcome by the pressure of the sebum layer, because the gland is still able to empty its excretion into the follicular canal, although no material arrives at the surface. It is rather, as Dünner states, a simple brake mechanism or a friction resistance which acts against the outflow of sebum to the surface.

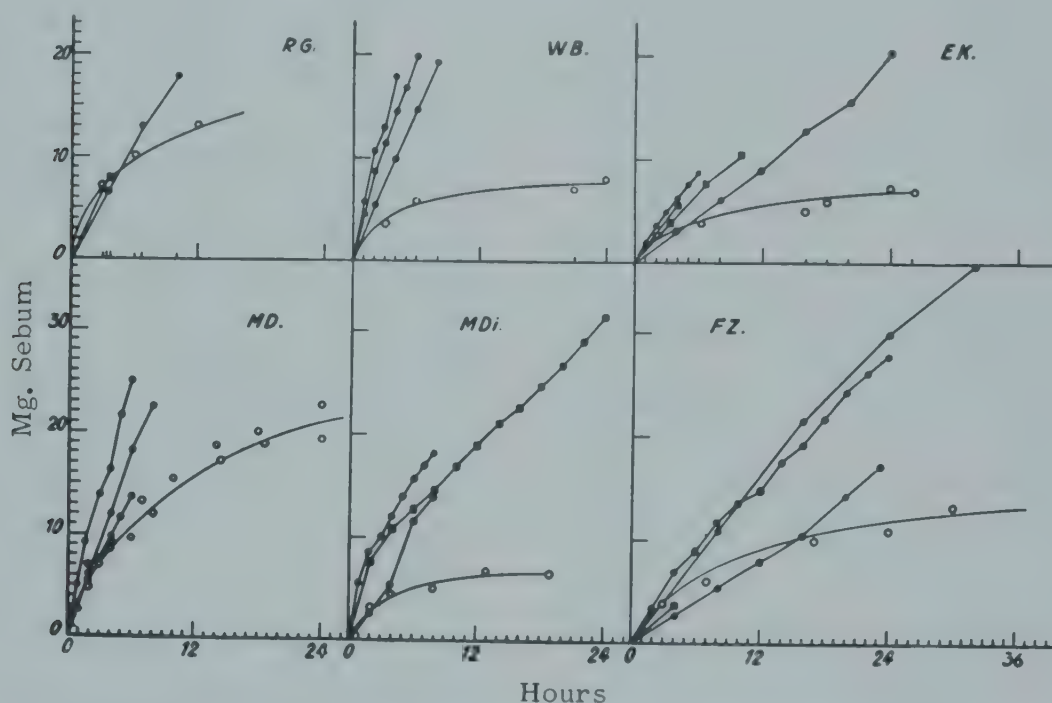


FIG. 6.—Excretion rate and saturation level in six subjects. Dot-dash lines indicate excretion rate; dash-circle lines indicate saturation level. All values obtained from 40 sq. cm. of surface of forehead at room temperature. From Zehender and Dünner (85). (Reproduced by permission of S. Karger.)

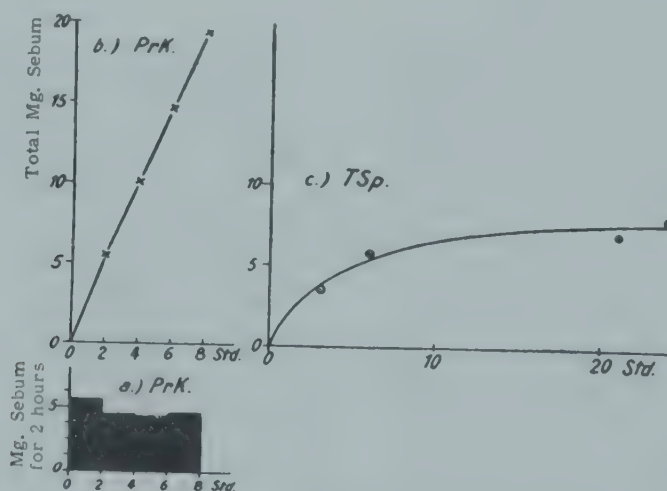


FIG. 7.—Comparison of excretion rate and saturation level. *PrK*, excretion rate; *TSp*, saturation level; *Std*, time in hours. From Zehender and Dünner (85). (Reproduced by permission of S. Karger.)

Butcher and Parnell (12) came in all essential points to the same conclusions as the foregoing. These authors demonstrated that the constancy of the saturation level is not due to a dynamic equilibrium in which sebum would be either volatilized or absorbed from the surface and replaced by newly excreted material but that, actually, expulsion to the surface ceases because of the resistance of the surface layer. They also found that artificial variation of the surface tension of sebum has no influence on the saturation mechanism.

There has never been any clarification of what would occur if frozen sebum occluded the pores permanently, as might happen in cold climates with no opportunity to wash for several weeks. If sebaceous-gland proliferation went on, there should be maximal dilatation of the follicles, with subsequent pathological changes. Apparently this does not happen. If the pore is occluded not by frozen sebum but by horny masses, pathological changes set in relatively early. This is the case in macro- and microscopic comedones and in different types of follicular keratoses. In the latter case the end-result of such absolute resistance to sebum excretion is known to be complete atrophy of sebaceous glands. Such effects caused by occlusion by frozen sebum have not been reported. The most likely explanation of the lack of severe changes in the cold is that sebaceous-gland proliferation decreases or stops entirely if sebum cannot be expelled to the surface. Miescher (48), after recording extremely low saturation levels in the high Alps, observed that such low levels persisted for a while after returning to lowlands. It seemed that the occlusion forced the glands to decrease their function and that several days were needed for readjustment. Butcher and Parnell (12), too, are inclined to assume that "when the sebum on the skin is not removed, cell proliferation within the gland probably progresses at a low level."

By applying a novel method of lipid estimation on the surface, based on the spreading properties of fat on a water surface in monomolecular layers (36, 37), Jones, Spen-

cer, and Sanchez (38) have arrived at a somewhat different interpretation of saturation level and secretion rate. They find that the accumulation of fat at the surface is irregular and that the main reasons for this irregularity are different rates of spreading and contamination of the area in which measurements are made with fat from neighboring skin. It is difficult, if not impossible, to isolate an area so that surface fat cannot flow in and out. The authors find that the main factor in influencing the rate of spreading is moisture of the skin surface. The difference is indeed amazing. The spread is 140 times greater on moist skin in 15 seconds than it is in 10 minutes on dry skin. The fat flows with a speed of 3.3 cm/sec over wet skin, while its flow over dry skin is negligible and close to zero. The authors believe that these facts make "the determination of the rate of secretion of a given area not only difficult but of mere academic importance. . . . Fat undoubtedly flows from areas of high secretion . . . to areas of low secretion."

The work of Jones *et al.* was done with a precision hitherto unequaled in this field, and their criticism of previously used techniques is fully justified. Still it should be admitted that the fundamental regularity of a parabolic decrease of fat excretion could be established with coarser methods and basically has not been refuted by the authors. The main difference between their results and earlier work is that at saturation level a minimal excretion continues to occur. This, of course, may depend, among other things, on the environmental temperature, a factor the effect of which was not investigated by Jones *et al.*

The most important finding in Jones's work is the tremendous influence of moisture on the spreading of the fatty film. This finding fits well with the work of Herrmann and Prose (28) and of Herrmann, Prose, and Sulzberger (29). They showed that regional variations in the level of ether-soluble substances strikingly paralleled the regional variations in sweat delivery. Wherever there was a difference of sebum levels in sym-

metrical sites, a corresponding difference was found also in sweating ability. On the palms these authors were able to increase the rate of fat excretion one and one-half fold when they stimulated sweat secretion by dry heat. Sweat, by increasing the spread of the lipid film, indirectly increased the amount of lipids appearing on the surface. The authors pointed out the significance of these findings for the pathology of common dermatoses and particularly for "dry skin"; anomalies of sweat secretion and fat excretion are closely interwoven, and no isolated anomaly can be postulated either in "dry

of Jones *et al.* (38) and Herrmann and Prose (28) on the decisive role of moisture, it appears that quantitative data from experiments in which these factors were not controlled are of little value. In addition, practically all methods hitherto employed have inherent imperfections (38). Still, it might be of some interest to present the data available after recalculation to equivalent terms, to indicate the range of magnitude with which we have to deal (see Table 2).

It would be a hopeless task to try to compute from the data in Table 2 the fat excre-

TABLE 2
SATURATION LEVEL (γ /SQ CM) AND EXCRETION RATE (γ /SQ CM/MIN)
OF THE FATTY FILM ON HUMAN FOREHEAD

Author	Satura- tion Level	Excre- tion Rate	Remarks	Method
Rabbeno (62).....	401	At 10°-15° C. room temperature	Paper absorption
Serrati (73).....	385	At 16°-18° C. room temperature	Paper absorption
Miescher and Schönberg (48)....	445	0.88		Paper absorption
Kirk (41).....	289	.41	Mean of 234 estimates in men and women 16-60 years old	Cup
Butcher and Parnell (12).....	160	One individual	Cup
Dünner (15).....	330	.48		Paper absorption
Kvorning (44).....	171	.53	14 women, 20-73 years old	Cup
Kvorning (44).....	223	.76	27 men, 22-60 years old	Cup
Kvorning (44).....	176	.70	9 women, 20-39 years old	Cup
Kvorning (44).....	186	0.77	13 men, 20-39 years old	Cup
Herrmann and Prose (28).....	338		Cup

skin" or in such common dermatoses as seborrheic dermatitis, atopic dermatitis, or ichthyosis (see p. 225).

Miescher (48) calculated that the thickness of the frozen sebum layer at saturation level varies between 3 and 10 μ . However, he emphasized that the layer must actually be thinner than calculated from amounts of sebum per unit surface, because much of it lies in follicular pores and skin folds. Butcher and Parnell (12) demonstrated that sebum flows preferentially along the sulci. From the data of Jones *et al.* (38) much thinner layers can be calculated.

Since the work of Dünner (15) on the decisive role of air temperature and the work

tion of the whole body surface. The regional differences are great, and, in particular, the fat excretion on the forehead is several times greater than on trunk and extremities (p. 295). The variations caused by temperature, moisture, and removal of the fatty film, either casually or by washing, are even greater.

Jones *et al.* (38) established with great precision that the minimum excretion rate is 0.1 γ /sq cm/min. This would indicate that the minimum excretion of the whole body surface after defatting is in the range of 200 γ /min or 12 mg/hr. The actual excretion is probably much higher than this minimal value.

IV. REGIONAL DIFFERENCES

Most estimations were made on the forehead, because the sebaceous-gland excretion is most active in this area (19, 20, 44, 12, 28). As compared with trunk and extremities, all workers find considerably higher values on the forehead. Serrati (73), who compared the excretion of the forehead and on flexor sides of the forearm in hundreds of

in Figure 8. His data clearly show that sebaceous excretion is (1) greater on the trunk than on the extremities; (2) greater on the chest than on the back; (3) has a definite maximum in the mid-line of the trunk, with rapidly decreasing values toward the sides; (4) is greater on upper than on lower extremities; (5) is slightly greater on flexor

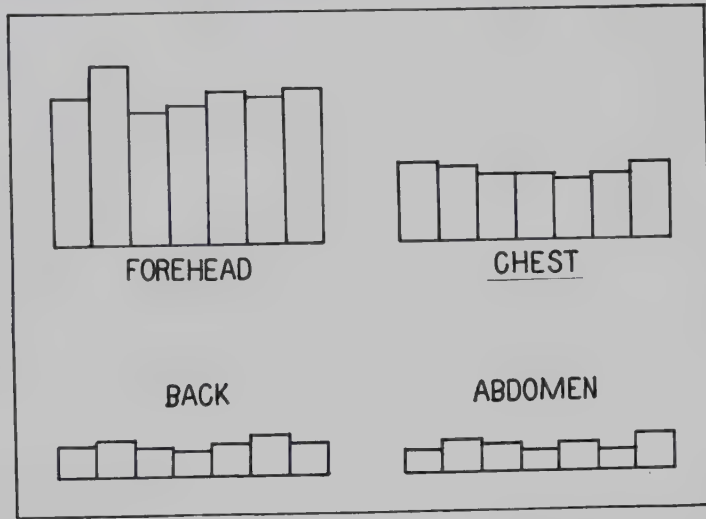


FIG. 8.—Constancy of sebum level. Determinations made in a single individual over a period of 7 days. Modified from Emanuel (19). (Reproduced by permission of Acta dermato-venereologica.)

estimations, finds in normals more than three times larger amounts on the forehead than on the forearm (an average of 15.4 mg. as against 4.2 mg/40 sq cm/12 hr). Comparative measurements on the forehead and other parts of the face and on the scalp are lacking.

Emanuel (19) investigated regional differences systematically. His results as obtained in one representative case are shown

than on extensor sides; and (6) is found in by far the largest amounts on the forehead.

Emanuel's results were recently fully confirmed by Herrmann and Prose (28). The distribution of lipid excretion conspicuously parallels the regional distribution of sweating ability (28, 29). In some instances an asymmetrical distribution of the amount of lipids was found on the right and left sides of the forehead (15, 28).

V. INFLUENCE OF AGE AND SEX

It has long been known that the amount of fat excreted to the skin surface is small in childhood, increases rapidly with oncoming puberty, and then levels out to fairly constant values at the end of puberty or shortly thereafter (68). The old data have been confirmed many times.

Emanuel (19) found that in children the amount of fat is about one-third that in

adults all over the body surface. Interestingly, however, he finds high values—in the range of that of adults—in the newborn, also after thorough removal of vernix caseosa.

Kvorning (44) finds a moderate and gradual increase with the onset of puberty. His data, as well as those of Kligman and Ginsberg (42), show that stationary adult values are reached only between twenty and

twenty-five years of age, an interesting finding in connection with the frequent persistence of acne vulgaris in this age group. The data of Kligman and Ginsberg (42) and of Nicolaides and Rothman (54) on the amount of hair fat in different age groups are

TABLE 3
FAT CONTENT OF HAIR FROM
VARIOUS AGE PERIODS (42)

No. of Samples	Age Period	Per Cent Hair Fat
16.....	4- 7	1.76
14.....	7-10	1.52
17.....	11-13	4.65
13.....	15-25	4.21
19.....	26-35	5.37

given in Tables 3 and 4. Both tables reflect the approximately threefold increase in the amount of fat in adolescence.

but he emphasized that individual variations are much too great to justify any decisive conclusion. A moderately greater amount of sebum in men seems to appear also in the data of Kvorning (44) and—to a lesser degree—in those of Kirk (41). The statistical significance of the slight differences has never been calculated, and it has not been claimed that they are significant. Perhaps, with better standardization of the methods, it will be possible to arrive at more definite conclusions.

While sex differences in the adult before senescence are debatable, a conspicuous sex difference seems to occur in old age. From the age of seventy on, men do not show any characteristic change in sebaceous excretion. Suzuki (76) and Kvorning (44) find some decrease, and Kirk (41) some tendency to increase. In contrast, *in women there is a significant drop in old age.* This was first discovered by Suzuki (76), who obtained values

TABLE 4
FAT CONTENT OF HUMAN HAIR IN DIFFERENT GROUPS (54)

Sex	Age	Race	No. of Samples	No. of Hair Cuts*	Percentage Range	Per Cent Fat in Hair†
Male.....	6-12	White	5	148	1.18-1.93	1.64
Male.....	15-20	White	1	31	4.76
Male.....	"Adult"	White	5	860	3.78-5.01	4.49
Male.....	20-40	White	1	61	4.50
Male.....	40-65	White	1	52	4.78
Male.....	Above 65	White	1	52	4.83
Male.....	"Adult"	Negro	3	755	7.50-8.59	7.71
Female.....	"Adult"	White	4	790	4.47-4.92	4.54
Female.....	Above 65	White	1	115	4.00
Female.....	"Adult"	Negro	2	55	6.74-7.13	7.07

* Computed on the basis of 5 gm. hair per hair cut in all the pooled samples.
† Values given are averages weighted according to sample size.

No consistent sex differences have been demonstrated in childhood, puberty, and the average adult age. Relatively most pronounced differences were found by Suzuki (76), who claimed that, with oncoming puberty, sebum excretion becomes much higher in the male than in the female and stays higher throughout adult life. Emanuel (19), too, had the general impression that sebum excretion is greater in men than in women;

in old women which fell below values obtained in prepubertal girls. Similarly impressive data were obtained by Kirk (41), whose data are summarized in Table 5 and Figure 9. The same tendency can be recognized in the data of Nicolaides and Rothman in Table 4.
The percentages given in Table 4 are a rough index of the thickness of the oily film on the hair. It shows the threefold increase

of the amount of hair fat with the advent of puberty and the tendency toward diminution in postmenopausal women. Men seem to show a small increase with advancing age. Both these changes in senility, how-

white adults. Negro men showed slightly higher values than Negro women; but again the significance of these findings remains to be established. Differences between white and Negro adults were quite pronounced,

TABLE 5
SUMMARY OF MEAN TOTAL LIPID SECRETION VALUES FROM 10-SQ. CM.
AREA OF FOREHEAD IN 125 MEN AND 109 WOMEN (41)

AGE GROUP	MEN			WOMEN		
	12-Hour Period (Mg.)	4-Hour Period (Mg.)	4-Hour Period in Percentage of 12-Hour Period	12-Hour Period (Mg.)	4-Hour Period (Mg.)	4-Hour Period in Percentage of 12-Hour Period
16-39.....	2.80	2.24	83	3.02	2.30	76
40-59.....	3.03	2.45	91	2.59	1.76	76
60-69.....	2.84	2.05	83	2.83	1.76	59
70-79.....	3.34	2.24	69	1.66	0.92	53
Above 80....	3.30	2.15	62	1.23	0.61	46

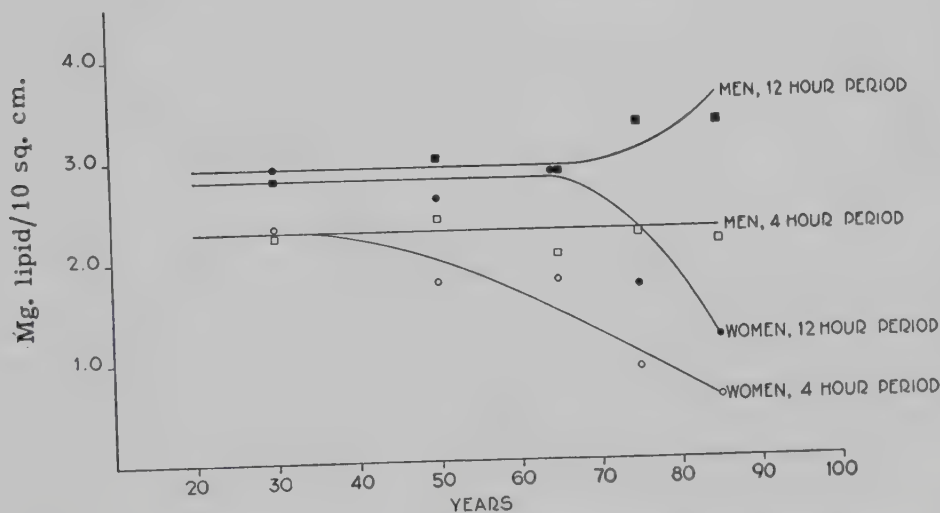


FIG. 9.—Total lipid secretion on 10 sq. cm. of skin of forehead in different sex and age groups (234 individuals). From Kirk (41). (Reproduced by permission of the author.)

ever, require further corroboration. No sex differences have been found by Nicolaides and Rothman in the hair fat content of

Negro men having 70 per cent more hair fat than white men, and Negro women about 60 per cent more than white women.

VI. ENDOCRINE INFLUENCES

The increase of sebaceous-gland excretion with oncoming puberty is accompanied and obviously caused by an increase in sebaceous-gland volume. The main event is en-

largement of lobules and possibly formation of new lobules but no increase in the size of single cells (16, 17, 27). The volume increase is so conspicuous that it can be recognized

qualitatively by comparing histological skin sections of children and adults. The pubertal stimulus acts by promoting mitotic division of the basal cells in the glands (48, 16, 17). (see p. 290). The stimulus is equally, or almost equally, effective in males and females (p. 299).

Like other pubertal changes, such as development of axillary and pubic hair in both sexes, development of mammary glands in the female, development of beard, body hair, and laryngeal changes in the male, pubertal development of sebaceous glands reaches a maximum some time after pu-

acne if they are treated with large doses of testosterone. This hormone is particularly effective in producing acne when it is given to persons who have a genetic predisposition to this disease.

The first two points were carefully studied by Hamilton (24, 25) on a great number of prepubertal castrates and eunuchoids. One can conclude from his observations not only that such individuals are free of seborrhea and acne but also that their sebaceous glands do not show any sign of pubertal development. He states in his description:

... Touching of the skin with the finger tips sufficed to indicate that the hair and scalp, like the facial skin, were less oily than in normal men of comparable age. From the scalp washed twice with ether, the following quantity of ether-soluble material was obtained: 971 mg. from M. B., 624 mg. from O. R., 834 mg. from N. W. These quantities were less than those obtained from any of three normal males 18, 25 and 30 years of age, the average recovery from whom was 1,334 mg. Both in number and size the fluorescent points at follicular orifices... on the scalp of sexually immature men were much less than in normal male relatives or in other normal adult men.

In contrast, among the 34 men who were castrated later, namely, when fourteen to nineteen years of age, excess sebaceous secretion was present in many of them but less than in normal men of their age (25).

Administration of testosterone propionate to normal prepubertal male children causes easily recognizable sebaceous-gland hyperplasia (64). In animal experimentation similar effects were observed in the rat (23, 16), in the hamster (26), and in the rabbit (63). Quantitative measurements of this effect were made by Ebling (16). His data (Table 6) clearly show what a powerful growth stimulus this hormone exerts on sebaceous glands. An even greater effect was demonstrated recently by Haskin *et al.* (27) in my laboratory. When testosterone propionate was given to rats in doses of 1 mg. daily for 30 days, the average increase of sebaceous-gland volume was about 400 per cent (Fig. 10).

TABLE 6

INFLUENCE OF SEX HORMONES ON VOLUME OF SEBACEOUS GLANDS AND ALVEOLI THEREOF IN THE RAT (EBLING) (16)
(Average Figures)

Treatment*	Sebaceous Cell Count	Alveolar Count	Cell Count per Alveolus
None.....	277.6	37.1	7.5
Estradiol benzoate 1 γ	178.3	28.1	6.3
Estradiol benzoate 100 γ	90.7	23.8	3.7
Testosterone propionate 1 mg.....	580.0	46.6	12.4

* Daily injections for 30 days.

berty, and then no further growth of the glands takes place. Some kind of equilibrium is established, although the endocrine stimulation persists. Pubertal development is the most obvious evidence that the normal development of sebaceous glands and maintenance of their function are under endocrine influence.

In the male the role of testicular hormones has long been recognized, mainly on the basis of three observations: (1) prepubertal castrate and eunuchoid males do not develop seborrhea or acne, a disease which is invariably connected with sebaceous-gland hyperplasia (65, 24); (2) castrate and eunuchoid males develop acne if they are treated with testosterone (24); and (3) normal males and females may develop

While in the male the pubertal development of the sebaceous glands is related beyond doubt to the production of testicular hormone, the similar development in the female has remained unexplained until most recently (p. 300). The identical, or almost identical, amount of sebum excreted in males and females (p. 296), as well as the approximately 1:1 sex incidence of acne

(6), makes it obvious that there must be some factor in the female which is comparable to the effect of testicular hormones in the male. It was assumed that, in girls, increased androgens produced by the adrenals during puberty are responsible for sebaceous-gland hyperfunction, particularly because all the urinary 17-ketosteroids in the female originate from the adrenal cortex

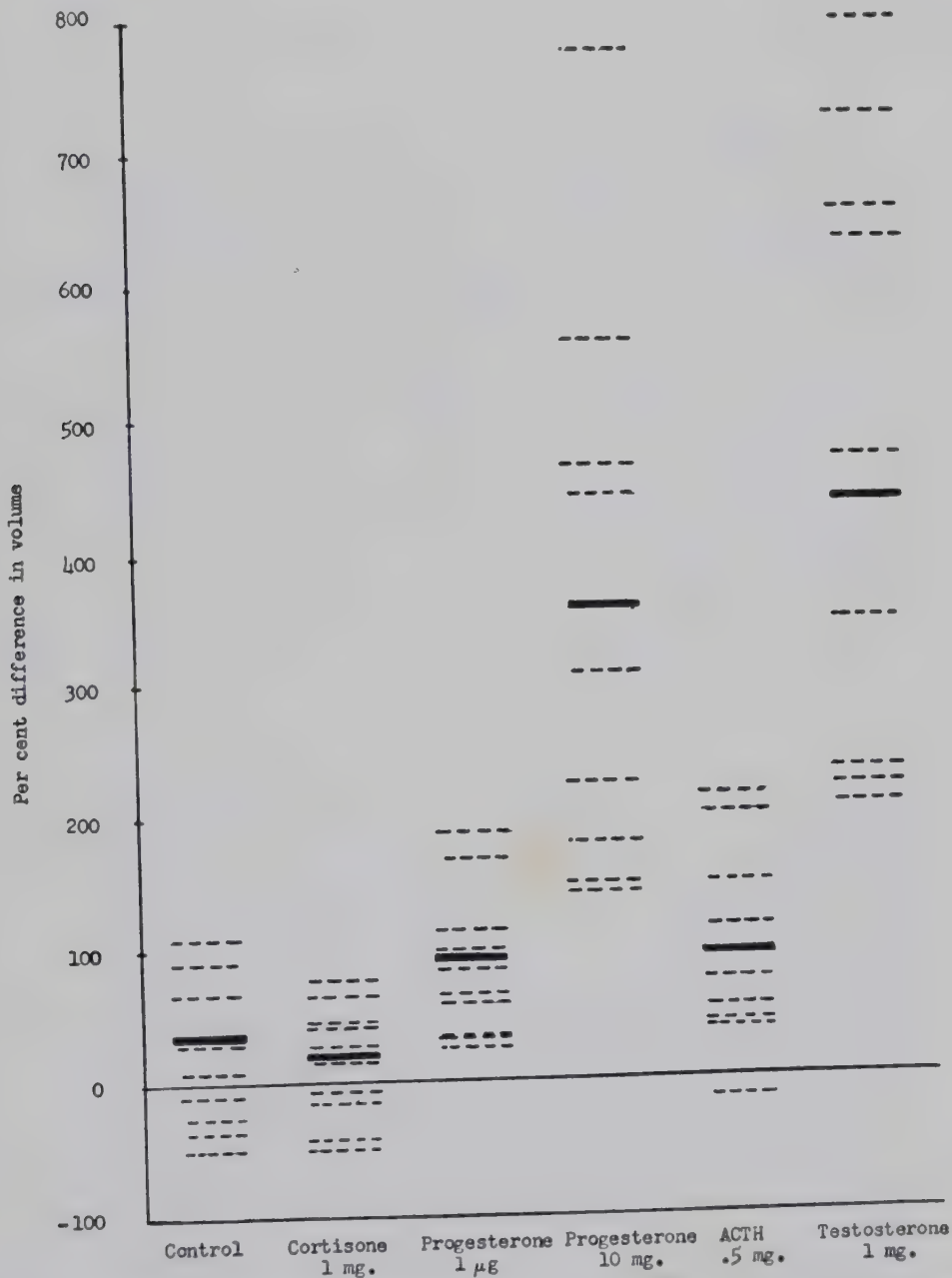


FIG. 10.—The effect of hormones on the sebaceous-gland volume in ovariectomized white rats. Change in volume after hormone treatment expressed in percentages. Dashed line represents individual rat; solid line represents average for 10 rats. From Haskin, Lasher, and Rothman (27). (Reproduced by permission of the Williams & Wilkins Company.)

(21). It is difficult, however, to accept this view because male castrates and most ovarian-deficient females have well-functioning adrenal glands and yet do not develop seborrhea or acne.

Soon after the advent of therapy with adrenocorticotropin and cortisone, it was observed that many patients of all age groups, young children included, developed acneform eruptions. Similar eruptions were known to occur also in Cushing's syndrome caused by hypercorticism. These acneform eruptions, however, are not identical with juvenile acne, because they lack the usual seborrhea and comedones. Natural juvenile acne has two main pathogenetic factors. One is sebaceous-gland hyperplasia, and the other is excessive follicular keratinization at the orifice, which occludes the pore and hinders the expulsion of sebum. If the follicular keratinization anomaly prevails, the acneform eruptions are papular and pustular but lack the greasy appearance of the skin that is seen in juvenile acne. In acneform eruptions developing in hypercorticism the major component is, clinically and histologically, follicular keratinization, and there is little, if any, sebaceous-gland hyperfunction (66). Thus these observations also fail to support an adrenal cortical origin for sebaceous-gland stimulation.

Local application of C-11 oxygenated steroids has resulted in inconsistent reduction in the size of rat sebaceous glands (4). Similar findings were noted upon parenteral administration of adrenocorticotropic hormone (3). That cortisone actually causes sebaceous-gland atrophy rather than hyperplasia was shown quantitatively by Haskin *et al.* (27). The average volume of sebaceous glands in the rat after daily injections of 1.0 mg. cortisone for 30 days was smaller than that of nontreated controls (Fig. 10). The results of the same authors with adrenocorticotropin have been inconclusive.

In the search for the hormone which is responsible for pubertal development of sebaceous glands in the female, the corpus luteum hormone was not thought to be of any

significance, particularly on the basis of findings of Ebling (16, 17), who elicited no effect on sebaceous glands after administration of 0.1 and 0.5 mg. progesterone daily for 30 days in rats. Recently, however, Haskin *et al.* (27) demonstrated a powerful effect of this hormone. Progesterone, 10 mg. daily for 15 days, produced in the rat an enlargement in volume of sebaceous glands comparable to that induced by testosterone, i.e., an average increase of 360 per cent. Even as small doses as 1 γ daily for 30 days produced an average increase in gland size of about 80 per cent (Fig. 10). The disagreement in the findings of Ebling (16, 17) and of Haskin *et al.* (27) has remained unexplained. Prior to the work of both, a moderately stimulating effect of progesterone was demonstrated by De Graaf (22, 23) on castrate male guinea pigs.

Because of the powerful effect of progesterone, as found by Haskin *et al.*, it was hypothesized (27) that pubertal development of sebaceous glands and occurrence of seborrhea and acne in the female depend on the production of corpus luteum hormone in the same way as they depend on the production of testicular hormone in the male. This hypothesis, since it does not implicate the adrenals, is consistent with the observation that hypogenital males and females do not develop seborrhea and acne.

The final proof of the validity of this hypothesis will be the experimental production of seborrhea and acne by the administration of large doses of progesterone in women in about the same percentage, as it is possible to do with testosterone.³ Also it should be shown that diffuse luteinization of the ovaries causes sebaceous-gland hyperplasia and predisposes to seborrhea and acne. There is one statement in the literature supporting the view on the role of luteinizing hormone. Selye (72) reports that the so-

3. Apparently, the development of acne under the influence of progesterone therapy was observed by Aron-Brunetière (R. Aron-Brunetière, *Essai d'interprétation physiopathologique de la séborrhée et de l'acné vulgaire*, *Excerpta med.*, Sec. XIII, 6 : 343, 1952).

called "lipid-cell tumors" of the ovaries, some of which are regarded as being connected with increased production of corpus luteum hormone, are often associated with the development of acne, which disappears after the surgical removal of the tumor. However, it has not been determined whether these tumors are true and pure "luteomas" rather than adrenal rest tumors.

Nothing is known about the influence of estrogenic hormones on sebaceous glands under physiological conditions (22, 23). The

administration of large nonphysiological doses, however, causes marked suppression of their growth. Hooker and Pfeiffer (30) found that this effect is counteracted in rats more in the male than in the female by the administration of testosterone propionate. While Hooker and Pfeiffer used doses which caused gross regression of all elements of epidermis and corium and particularly of follicles and hairs, Ebling (16) demonstrated an apparently selective atrophy of sebaceous glands with smaller doses (Fig. 11). Ebling

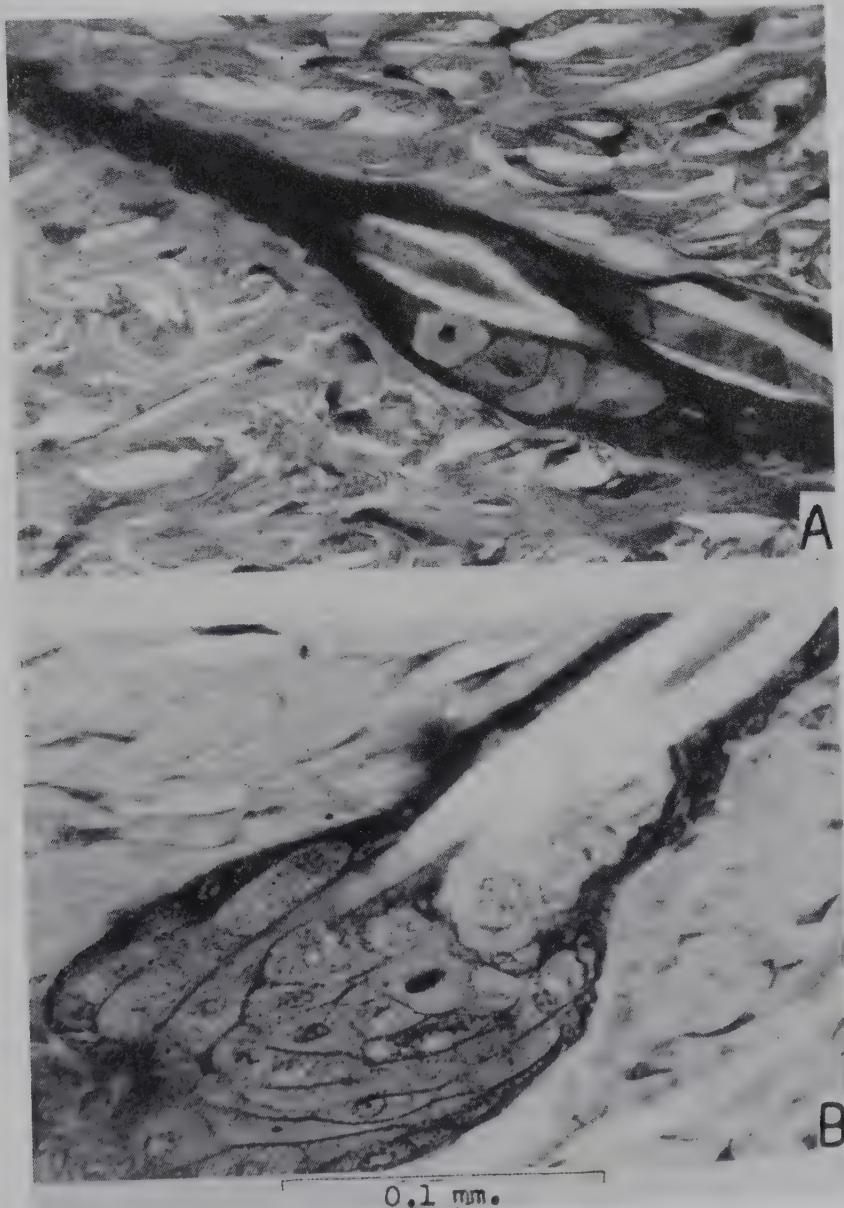


FIG. 11.—A, sebaceous gland of female white rat treated with 100 γ per day of estradiol benzoate for 36 days; B, sebaceous gland of female white rat treated with 1 mg. of testosterone propionate per day for 36 days. From Ebling (16). (Reproduced by permission of the Journal of Endocrinology.)

(17), on implantation of ovaries in immature rats, found an initial short period of volume increase of sebaceous glands, followed by a subsequent and permanent decrease much below normal.

It should be emphasized that, in order to demonstrate the depressive action of estrogenic hormones, doses must be used which are much greater than those used in clinical therapy. Whether doses of estrogenic hormone which can be given safely reduce the size of sebaceous glands appreciably is not known.

An interesting biphasic effect of estrogen administration was noted by Bullough (9) and by Ebling (16, 17). Estradiol benzoate causes a transient increase in the mitotic activity of the sebaceous glands. Mitotic activity reaches a peak in 2 days, then gradually declines to subnormal levels. Some initial stimulation was also noted by De Graaf (22).

From the available data one may conclude that the pubertal development of sebaceous glands is an effect of testicular hormone in the male and of progesterone in the female. Both exert a proliferative stimulus on the matrix cells of these glands. Thereby, the glands increase in size and produce more sebum than before. There is no increase in number of glands (64), and the size of the single cells does not change in this hyperplastic process (27). However, the alveoli of the glands enlarge greatly (64).

It might be worth while to mention that

some sebaceous glands completely atrophy if the sex hormone stimulation is eliminated. This is the case with the preputial glands of male mice, which are regarded as typical "sebaceous-gland organs" on the basis of their microscopic appearance and the composition of their excretion products (10). Voss (80) showed that these glands atrophy following castration and regenerate again on implantation of testicular tissue or on administration of male sex hormone.

It is also interesting to note that the male hormone is much more effective in stimulating sebaceous-gland development than it is in suppressing development of the mammary glands and of nipples (22). Conversely, it may be possible that the suppressive effect of estrogens on sebaceous glands is considerably weaker than its stimulating effect on mammary glands and nipples.

It was postulated that, in humans, abnormalities of sebaceous excretion leading to seborrhea and acne must be due to an abnormal ratio of androgens to estrogens (81, 45). Theoretically, this might very well be true. However, even greater variations in androgen-estrogen ratios may be found among eunuchoid and castrate men, who never have acne (24). Thus the significance of this hormone ratio in the pathogenesis of acne is debatable.

Little is known as to the influence of the thyroid gland. It seems that thyroidectomy in the young causes a regression of the pilosebaceous apparatus as a whole (39).

VII. NUTRITIONAL INFLUENCES

Forced feeding of animals with fats results in excretion of increased amounts of sebum, and the nutritional fat constituents are excreted unchanged (70, 43, 68). Obviously, this is an unphysiological situation in which the overloading of the organism makes it impossible to metabolize fat constituents physiologically. Quantitative measurements on the increase of sebum excretion under forced feeding were carried out on rabbits by Suzuki (76). When fat was given in excess, the amount of sebum in-

creased by 70–119 per cent within 2 weeks. In comparable overfeeding with carbohydrates the increase was only 11–38 per cent. Interestingly, when the abnormal type of feeding was continued, there was a tendency for the amount of sebum to decline again. This was interpreted as a sign of adaptation.

In humans, quantitative experiments on the influence of nutrition were made by Ser-rati (73) on normal persons and on acne patients. The amount of total lipids from a 40-sq. cm. area of the forehead 12 hours

after thorough defatting was 10.9–17.9 mg., with an average of 15.4 mg. in twelve normal individuals. When normal persons were first kept on a normal mixed diet and subsequently were given excess fat and later excess carbohydrates, there was a definite increase beyond the normal range with both types of diet, to approximately the same degree (Table 7).

In acne patients kept on a mixed regular diet the values of the sebum level were between 24.5 and 32.8 mg., with an average of 29 mg. in five individuals. When they were subsequently given a diet rich in fat and later a diet rich in carbohydrates, the first diet caused a moderate, the second diet—in two out of three cases—a much greater, in-

sistency toward a more liquid state and not necessarily an increase in excretion rate. The creation of a relatively liquid sebum, although causing the skin to appear more shiny, would otherwise not necessarily have an unfavorable effect on acne, because it may counteract inspissation and stagnation of sebum in the follicular canal.

Eckstein (18) found no change in the cholesterol content of hair fat in the rat on a diet rich in fat. He stated that this behavior is in contrast to that of the liver.

TABLE 8*

INFLUENCE OF DIET ON SEBUM LEVEL
IN ACNE PATIENTS (73)

Name	Age	Regular Diet	Excess Fat Intake	Excess Carbohydrate Intake
S. C.....	19	24.5	38.9	34.9
J. F.....	24	29.7	33.9	38.1
B. S.....	25	32.8	37.8	41.3

* Sebum level expressed in milligrams per 40 sq. cm. per 12 hours.

TABLE 7*

INFLUENCE OF DIET ON SEBUM LEVEL
IN NORMALS (73)

Name	Age	Regular Diet	Excess Fat Intake	Excess Carbohydrate Intake
F. M.....	21	17.2	21.7	22.1
L. B.....	27	16.8	20.5	20.9
M. B.....	25	17.9	22.0	24.4

* Sebum level expressed in milligrams per 40 sq. cm. per 12 hours.

crease in the sebum level (Table 8). These data are indeed impressive, showing a pronounced influence of excess carbohydrate intake on the sebum level in acne patients. The clinical implications are obvious. Therefore, it would be highly desirable to try to duplicate these results. Even more important would be the investigation, under similar conditions, of the excretion rate of sebum. Its increase in Serrati's experiments may indicate only a change in the con-

The possible influence of diet was often connected with the question concerning the constituents from which the sebum lipids are built. Histological evidence rather strongly suggests that they derive from the protoplasm of the sebaceous-gland basal cells. The composition of this protoplasm probably cannot vary to a great extent without danger to its viability. As discussed elsewhere (p. 316), the probability is that constituents of sebum are built from the small carbon fragments which are formed in catabolic processes by the cells. If a nutritional influence, as reported above, actually exists, their action must be a rather complicated one.

VIII. THE PROBLEM OF INNERVATION

There is a great deal of morphological evidence that sebaceous glands in man are supplied by nerves going both to the surrounding connective tissue and to the gland proper (77, 52, 79, 7, 34, 35, 14). A ramifying bundle of nerve fibers forms coarse nets in

the periglandular connective tissue. Their slender branches penetrate into the interlobular septae. There they break up into very fine fibers and form a plexus on the basement membrane of the gland. However, whether these unmyelinated (sympathetic?)

fibers actually enter the parenchyma of the gland and, if they do, whether they are true "secretory" fibers, the stimulation of which increases glandular activity, is a matter of controversy. Direct evidence for secretory innervation of sebaceous glands is certainly lacking up to the present.

When in the 1920's the first cases of post-encephalitic seborrhea were observed, the theory was set forth that sebaceous-gland innervation has an inhibitory center in the diencephalon and that, if this center is destroyed, the inhibition is released and flow of sebum results (74, 75). This theory was supported by experiments of Perutz, Lustig, and Klein (57). After intramuscular injections of oil into rabbits, they obtained a greater cutaneous excretion of fat in animals whose mesodiencephalon had previously been destroyed than they did in normal animals. However, this attractive theory suffered an unexpected blow when Miescher and Schönberg (48) showed that in post-encephalitic seborrhea the increase in sebum output is caused by glandular hyperplasia, the increase being directly proportional to the increase in excretory volume, in the same fashion as is the case in juvenile seborrhea (see Table 1 on p. 290). How the encephalitic process leads to glandular hyperplasia is not understood. So far, only hormones are known to influence the size of sebaceous glands, and it is feasible that the encephalitic process disturbs hormonal balance via the nerve tracts leading to the pituitary.⁴ Be that as it may, the findings of Miescher have shattered the foundation of the belief in a central nervous representation of sebaceous excretion.

The first and only attempt to demonstrate secretory innervation of sebaceous glands in physiological experimentation goes back to the last century, when Arloing (1, 2) electrically stimulated the cervical sympathetic in donkeys and produced prolific

fat secretion in the ear. This finding was never re-examined. Should it be duplicated, it will still have to be decided whether the stimulated glands are sebaceous glands or apocrine glands, which occur in the ear canal and which are known to have sympathetic innervation (p. 183). Another possibility would be that eccrine glands were stimulated and that eccrine sweat, emulsifying sebum, promoted sebaceous excretion.

Further evidence for the secretory innervation of sebaceous glands is of a clinical nature and not conclusive, partly because "increased sebum excretion" was established purely on the basis of inspection, without measurements, and partly because cases in which measurements were carried out concern central and peripheral nerve diseases in which vasomotor and sudomotor disturbances accompanied the increased excretion of fat; and therefore indirect action, particularly that of sweat, cannot be excluded.

The first often cited clinical observation of von Marschalko (46) concerns a patient who, after an injury with complete severance of the supraorbital nerve, displayed profuse sebum flow in the area supplied by this nerve. The flow of sebum ceased after the nerve was sutured. A similar observation was reported by Bujadoux (8). In both cases overflow of sebum was associated with hyperesthesia. The presence or absence of hyperhidrosis was not mentioned.

Unilateral development of acne and seborrhea subsequent to facial palsy on the affected side was reported by Truffi (79) and by Nexmand (53). These are, indeed, remarkable cases. But, as Nexmand pointed out, all phenomena could be interpreted by indirect influences, without assuming a secretory innervation of the sebaceous glands.

A monumental work dedicated to the problem of the innervation of sebaceous glands is that of Serrati (73). Working with the filter-paper absorption method, he carefully established normal values on forehead and forearm and subsequently carried out measurements on 38 patients suffering from a variety of nerve diseases. In 13 patients

4. Recent experiments in my laboratory indirectly support this view (N. Lasher, A. L. Lorincz, and S. Rothman, Hormonal effects on sebaceous glands in the white rat. II. The effect of the pituitary-adrenal axis, *J. Invest. Dermat.*, 22:25-31, 1954).

with postencephalitic seborrhea he found three to ten times increased sebum levels on the forehead, face, and arms. Apparently, there was an increase all over the body surface. In cases of one-sided extrapyramidal damage the flow of sebum was consistently greater on the more involved side. In one case of unilateral Parkinsonism the fat levels on the two sides differed as much as 4:1 on the forearm and as much as 5:1 on the forehead. That in these cases hyperidrosis may have contributed to the increase of sebum level can be suspected from the note that in 10 out of 13 cases the administration of belladonna reduced the seborrhea considerably.

Similarly increased saturation levels were found in cases of hemiplegia (73) and in pontobulbar tumors (73, 60) on the diseased side, and even in trigeminal and sciatic neuralgia (73). The main objection to the conclusion that such observations would prove the existence of secretory nerves to the sebaceous glands is the intricate interdependence with sweat secretion (pp. 160 and 293). Probably in all pathological cases cited here, changes in sweat secretion and to a lesser degree in vasomotor innervation satisfactorily explain the changes in the amounts of skin surface fats.

The problem of sebaceous-gland innervation has lately reappeared on the scene with the claim that emotions increase sebaceous-gland activity, so as possibly to imply direct nervous action (82, 83, 84, 40). It was

pointed out (67) that all these findings can be well explained by emotional hyperidrosis and that, as long as these results are not reproduced in atropinized individuals, they cannot be accepted as proof of the secretory innervation of sebaceous glands and of their having cortical representation. The claim that emotions cause the sebum to flow is by no means new (13).

An attempt to prove in another, indirect, way the existence of a secretory innervation of sebaceous glands, namely, by the action of drugs acting on the autonomic nervous system, has often been made. The older literature was reviewed by Cerutti (13). On the basis of histochemical findings, Melczer and Deme (47) claimed that pilocarpine stimulates sebaceous-gland activity (p. 331); but others could not find such an effect (67). Doupe and Sharp found that denervation of a skin area in man has no effect whatsoever on sebaceous excretion.⁵

The existence of stimulating and inhibitory nerve impulses to the sebaceous-gland cells will be proved satisfactorily only by physiological experimentation. As long as sudomotor and vasomotor influences are not excluded with certainty in the stimulation of certain centers and/or pathways, no conclusions can be drawn.

5. J. Doupe and M. E. Sharp, Studies in denervation. G. Sebaceous secretion, *J. Neurol. & Psychiat., N.S.*, **6**:133-35, 1943.

BIBLIOGRAPHY

1. ARLOING, S. Des rapports fonctionnels du cordon sympathique cervical avec l'épiderme et les glandes, *Arch. de physiol. norm. et path.*, 5th ser., **3**:160-71, 1891. Quoted by ROTHMAN and SCHAAF (68).
2. ———. Nouvelle contribution à l'étude de la partie cervicale, du grand sympathique envisagée comme nerve sécrétoire chez les animaux solipèdes, *ibid.*, pp. 241-52. Quoted by ROTHMAN and SCHAAF (68).
3. BAKER, B. L.; INGLE, D. J.; LI, C. H.; and EVANS, H. M. Growth inhibition in the skin induced by parenteral administration of adrenocorticotropin, *Anat. Rec.*, **102**:313-31, 1948.
4. BAKER, B. L., and WHITAKER, W. L. Growth inhibition in the skin following direct application of adrenal cortical applications, *Anat. Rec.*, **102**:333-48, 1948.
5. BIEDERMANN, W. Vergleichende Physiologie des Integuments der Wirbeltiere. Die Hautsekretion, *Ergebn. d. Biol.*, **6**:426-558, 1930.
6. BLOCH, B. Metabolism, endocrine glands and skin diseases, with special reference to acne vulgaris and xanthoma, *Brit. J. Dermat.*, **43**:61-87, 1931.
7. BOEKE, J. Le plexus sympathique fondamental situé dans le tissu conjonctif et ses rapports avec les éléments glandulaires,

- Bull. d'histol. appliq. à la physiol., **11**:221-40, 1934.
8. BUJADOUX, A. Séborrhée fluente avec hyperesthésie cutanée d'origine, Lyon méd., **157**:355-57, 1936.
 9. BULLOUGH, W. S. Mitotic activity in the adult female mouse, *Mus musculus* L. A study of its relation to the oestrus cycle in normal and abnormal conditions, Phil. Trans. Roy. Soc. London, s.B, **231**:453-517, 1946.
 10. BUSCHKE, W. Die Hautdrüsenorgane (Harsersche Drüsen, Inguinaldrüsen, Präputialdrüsen, Analdrüsen, Kaudaldrüsen, Kieferdrüsen) der Laboratoriumsnagetiere und die Frage ihrer Abhängigkeit von den Geschlechtsdrüsen, Ztschr. f. Zellforsch. u. mikr. Anat., **18**:217-43, 1933.
 11. BUTCHER, E. O., and PARNELL, J. B. Sebaceous secretion on the human head, J. Invest. Dermat., **9**:67-74, 1947.
 12. ———. The distribution and factors influencing the amount of sebum on the skin of the forehead, *ibid.*, **10**:31-38, 1948.
 13. CERUTTI, P. Fisiologia degli annessi cutanei, Gior. ital. di dermat. e sif., **75**:112-39, 1934.
 14. CHAMPY, C.; COUJARD, A.; and COUJARD-CHAMPY, C. L'innervation sympathique des glandes, Acta anat. (Basel), **1**:233-83, 1945-46.
 15. DÜNNER, M. Der Einfluss physikalischer Faktoren (Druck, Temperatur) auf die Talgabsonderung des Menschen, Dermatologica, **93**:249-71, 1946.
 16. EBLING, F. J. Sebaceous glands. 1. The effect of sex hormones on the sebaceous glands of the female albino rat, J. Endocrinol., **5**:297-302, 1948.
 17. ———. Sebaceous glands. 2. Changes in the sebaceous glands following the implantation of oestradiol benzoate in the female albino rat, *ibid.*, **7**:288-98, 1951.
 18. ECKSTEIN, H. C. Sterol metabolism in young white rats. III. Effect of high and low fat diets on sterol content of hair, J. Biol. Chem., **125**:107-12, 1938.
 19. EMANUEL, S. Quantitative determination of the sebaceous glands' function, with particular mention of the method, Acta dermat.-venereol., **17**:444-56, 1936.
 20. ———. Mechanism of sebum secretion, *ibid.*, **19**:1-18, 1938.
 21. FRASER, R.; FORBES, A. P.; SULKOWITCH, H. W.; and REIFENSTEIN, E. C. Colorimetric assay of 17-ketosteroids in urine: survey of use of this test in endocrine investigation, diagnosis, and therapy, J. Clin. Endocrinol., **1**:234-56, 1941.
 22. GRAAF, H. J., DE. Endocrine influences on sebaceous glands, Acta brev. Neerland., **12**:67-68, 1942.
 23. ———. L'influence des hormones sexuelles sur les glandes sébacées et sur la peau, *ibid.*, **13**:77-78, 1942.
 24. HAMILTON, J. B. Male hormone substance: a prime factor in acne, J. Clin. Endocrinol., **1**:570-92, 1941.
 25. ———. Male hormone stimulation is prerequisite and an incitant in common baldness, Am. J. Anat., **71**:451-80, 1942.
 26. HAMILTON, J. B., and MONTAGNA, W. The sebaceous glands of the hamster. I. Morphological effects of androgens on integumentary structures, Am. J. Anat., **86**:191-234, 1950.
 27. HASKIN, D.; LASHER, N.; and ROTHMAN, S. Some effects of ACTH, cortisone, progesterone, and testosterone on sebaceous glands in the white rat, J. Invest. Dermat., **20**:207-12, 1953.
 28. HERRMANN, F., and PROSE, P. H. Studies on the ether-soluble substances on the human skin. I. Quantity and "replacement sum," J. Invest. Dermat., **16**:217-30, 1951.
 29. HERRMANN, F.; PROSE, P. H.; and SULZBERGER, M. B. Studies on sweating. V. Studies of quantity and distribution of thermogenic sweat delivery to the skin, J. Invest. Dermat., **18**:71-86, 1952.
 30. HOOKER, C. W., and PFEIFFER, C. A. Effects of sex hormones upon body growth, skin, hair and sebaceous glands in the rat, Endocrinology, **32**:69-76, 1943.
 31. HOU, H. C. Studies on the glandula uropygialis of birds, Chinese J. Physiol., **2**:345-80, 1928.
 32. ———. Relation of the preen gland (glandula uropygialis) of birds to rickets, *ibid.*, **3**:171-82, 1929.
 33. ———. Relation of preen gland of birds to rickets. III. Site of activation during irradiation, *ibid.*, **5**:11-18, 1931.
 34. ISTÓK, J. Nerve endings of sebaceous glands, Orvosi hetil. (Hungarian), **81**:348-49, 1937.
 35. JOHN, F. Zur vegetativen Innervation der Talgdrüsen, Arch. f. Dermat. u. Syph., **182**:402-11, 1942.
 36. JONES, K. K. A micromethod for fat

- analysis based on formation of monolayers, *Science*, **111**:9, 1950.
37. ———. A micromethod for fat analysis based on formation of monolayer films, *Quart. Bull., Northwestern Univ. M. School*, **24**:253–56, 1950.
 38. JONES, K. K.; SPENCER, M. C.; and SANCHEZ, S. A. The estimation of rate of secretion of sebum in man, *J. Invest. Dermat.*, **17**:213–26, 1951.
 39. JURENKA, W. Fettsekretion der Körperhaut und Schilddrüse. (Ein Beitrag zur Kenntnis des Einflusses der Schilddrüsenfunktion auf Wolle bzw. Haarkleid und Körperhaut einiger Haustiere mit besonderer Berücksichtigung ihrer Fettsekretion), *Ztschr. f. Zücht., Reihe B, Tierzücht. u. Züchtungsbiol.*, **31**:373–91, 1935.
 40. KEPECS, J. G., and ROBIN, M. The relationship between certain emotional states and the rate of secretion of sebum, *J. Invest. Dermat.*, **20**:373–84, 1953.
 41. KIRK, E. Quantitative determinations of the skin lipid secretion in middle-aged and old individuals, *J. Gerontol.*, **3**:251–66, 1948.
 42. KLIGMAN, A. M., and GINSBERG, D. Immunity of the adult scalp to infection with *Microsporum audouinii*, *J. Invest. Dermat.*, **14**:345–58, 1950.
 43. KLINGE, F., and WACKER, L. Über den Lipidstoffwechsel und die Gewebsveränderungen bei Mäusen und Kaninchen unter dem Einfluss von Fett-, Cholesterin- und Scharlachrotfütterung, *Krankheitsforsch.*, **1**:257–85, 1925.
 44. KVORNING, S. A. Investigations into the pharmacology of skin fats and ointments. II. On the occurrence and replenishment of fat on the skin in normal individuals, *Acta pharmacol. et toxicol.*, **5**:262–69, 1949.
 45. LAWRENCE, C. H., and WERTHESSEN, N. T. Treatment of acne with orally administered estrogens, *J. Clin. Endocrinol.*, **2**:636–38, 1942.
 46. MARSCHALKO, T. VON. Über einen eigentümlichen Fall von circumscripiter profuser Hauttalgsekretion (ein Beitrag zum Nerven einfluss auf die Hauttalgabsonderung?), *Dermat. Ztschr.*, **12**:713–18, 1905.
 47. MELCZER, N., and DEME, S. Beiträge zur Tätigkeit der menschlichen Talgdrüsen. II. Rolle und Formveränderungen des Golgi-Apparates während der Talgproduktion, *Arch. f. Dermat. u. Syph.*, **183**:388–95, 1942.
 48. MIESCHER, G., and SCHÖNBERG, A. Untersuchungen über die Funktion der Talgdrüsen, *Bull. schweiz. Akad. d. med. Wissensch.*, **1**:101–14, 1944.
 49. MONTAGNA, W., and NOBACK, C. R. Histochemical observations on sebaceous glands of rat, *Am. J. Anat.*, **81**:39–62, 1947.
 50. MONTAGNA, W.; NOBACK, C. R.; and ZAK, F. G. Pigment, lipids, and other substances in the glands of the external auditory meatus of man, *Am. J. Anat.*, **83**:409–36, 1948.
 51. MÜLLER, J., and REINBACH, H. Untersuchungen über das Hautsekret der Fische; die Chemie des Aalschleims, *Ztschr. f. physiol. Chem.*, **92**:56–74, 1914.
 52. NAKANISHI, K. A contribution to the histologic study of the nerve fibers in the sebaceous gland and the arrector muscle of hair of the human body, *Jap. J. Dermat. & Urol.*, **30**:1134–37, 1930.
 53. NEXMAND, P. H. Case of seborrhea with comedones in conjunction with facial paralysis, *Acta dermat.-venereol.*, **25**:275–80, 1944.
 54. NICOLAIDES, N., and ROTHMAN, S. Studies on the chemical composition of human hair fat. II. The overall composition with regard to age, sex and race, *J. Invest. Dermat.*, **21**:9–14, 1953.
 55. OKAJIMA, K., *et al.* Quantitative Untersuchungen der Anhangsorgane der Haut, *Folia anat. japon.*, **9**:215–61, 1931; **10**:125–68, 721–52, 753–92, 1932; **11**:41–84, 443–500, 501–52, 1933; **12**:65–97, 165–205, 229–90, 1934; **13**:63–78, 79–112, 477–90, 583–630, 1935; **14**:47–95, 1936; **15**:1–41, 59–149, 195–238, 1937; **16**:387–94, 1938.
 56. PARNELL, J. P. Postnatal development and functional histology of the sebaceous glands in the rat, *Am. J. Anat.*, **85**:47–71, 1949.
 57. PERUTZ, A.; LUSTIG, B.; and KLEIN, A. E. Zur zentralen Regulation des Fettstoffwechsels der Hautoberfläche, *Arch. f. Dermat. u. Syph.*, **170**:510–20, 1934.
 58. PETIT, A., and GEAY, F. Sur la glande cloacale du Caiman, *Bull. Mus. hist. nat. Paris*, **11**:112–13, 1905.
 59. PINKUS, F. Die normale Anatomie der Haut. [In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1/1**:1–378. Berlin: J. Springer, 1927.]
 60. PINTUS, G. Vasomotilità, sudore, minzione e secrezione sebacea nelle lesione ponto-bul-

- bari (contributo anatomoclinico), Riv. sper. di freniat., **62**:5-50, 1938.
61. PRITCHARD, J. E.; EDWARDS, L. D.; and CHRISTIAN, J. E. A study of the surface lipids of skin, J. Am. Pharm. A. (scient. ed.), **28**:546-49, 1949.
 62. RABBENO, A. Contributo allo studio della secrezione sebacea, Gior. ital. di mal. ven. pelle, **65**:1509-14, 1924.
 63. REIS, F., and GELLIS, S. Effects produced on the pilosebaceous system and the adrenals of the rabbit by inunction of sex hormones, J. Invest. Dermat., **12**:159-72, 1949.
 64. RONY, H. R., and ZAKON, S. J. Effect of androgens on the sebaceous glands of human skin, Arch. Dermat. & Syph., **48**:601-4, 1943.
 65. ROTHMAN, S. Das inkretogene Moment in der Dermatologie. In: BAYER and VON DEN VELDEN, Klinisches Lehrbuch der Inkretologie und Inkretotherapie, pp. 382-404. Leipzig: G. Thieme, 1924.
 66. ———. Discussion of paper by M. A. BRUNSTING, Hidradenitis and other variants of acne, Arch. Dermat. & Syph., **65**:303-15, 1952.
 67. ROTHMAN, S., and HERRMANN, F. Discussion of KEPECS and ROBIN (40).
 68. ROTHMAN, S., and SCHAAF, F. Chemie der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1/2**:161-377. Berlin: J. Springer, 1929.
 69. SCHAFFER, J. Zur Phylogenese der Talgdrüsen, Ztschr. f. mikr.-anat. Forsch., **22**:579-90, 1930.
 70. SCHMIDT, M. B. Über vitale Fettfärbung in Geweben und Sekreten durch Sudan und geschwülstartige Wucherungen der ausschließenden Drüsen, Virchows Arch. f. path. Anat., **253**:432-51, 1924.
 71. SCHUR, H., and GOLDFARB, L. Zur Physiologie und Pathologie des Talgsekretion. I. Untersuchungsmethodik und allgemeiner Sekretionsmechanismus, Wien. klin. Wchnschr., **40**:1255-59, 1927.
 72. SELYE, H. Textbook of endocrinology. With a Preface by B. A. HOUSSAY. 2d ed. Montreal: Acta endocrinologica, 1949.
 73. SERRATI, B. Influenza del systema nervosa sulla secrezione sebacea. Osservazioni e ricerche cliniche, Riv. di pat. nerv., **52**:377-423, 1938.
 74. STIEFLER, G. Die Seborrhoea faciei als ein Symptom der Encephalitis lethargica, Ztschr. f. d. ges. Neurol. u. Psychiat., **73**:455-63, 1921.
 75. ———. Seborrhoea faciei als isolierte post-enzephalitische Restveränderung, Wien. klin. Wchnschr., **37**:334-35, 1924.
 76. SUZUKI, S. Zur Physiologie und Pathologie der Talgsekretion, besonders bei Lues, Jap. J. Dermat. & Urol., **40**:203-13, 1936.
 77. TAKINO, M. Die Innervation der menschlichen Haut, besonders über die Musculi erectores pilorum, der Talgdrüsen, der Schweissdrüsen, und der kleinen Haare, Acta scholae med. univ. imp. Kioto, **12**:281-94, 1929.
 78. TRUFFI, G. Le applicazioni indirette di Raggi X nel campo della dermosifilopatia, Arch. ital. di dermat., sif., **9**:3-69, 1933.
 79. ———. Innervazione degli annessi cutanei, Arch. Ist. biochim. ital., **6**:409-42, 1934.
 80. VOSS, H. E. Die Präputialdrüsen der Maus in ihrer Abhängigkeit vom Hormon des Hodens, Ztschr. f. Zellforsch. u. mikr. Anat., **14**:200-221, 1932.
 81. WILE, U. J.; SNOW, J. S.; and BRODBURY, J. G. Studies of sex hormones in acne; urinary excretion of androgenic and estrogenic substances, Arch. Dermat. & Syph., **39**:200-210, 1939.
 82. WITTKOVER, E. Psychologic aspects of seborrhea, Bull. Menninger Clin., **11**:148-68, 1947.
 83. WITTKOVER, E., and MACKENNA, R. M. B. Psychologic aspects of seborrheic dermatitis, with foreword, Brit. J. Dermat., **59**:281-93, 1947.
 84. WOLF, S.; LORENZ, T. H.; and GRAHAM, D. P. The relation of life stress and emotions to human sebum secretion and to the mechanism of acne vulgaris, J. Lab. & Clin. Med., **41**:11-28, 1953.
 85. ZEHENDER, F., and DÜNNER, M. Untersuchungen über die Methoden zur Messung der menschlichen Hauttalgsekretion, Dermatologica, **93**:355-72, 1946.

CHAPTER 13

Composition of the Lipid Surface Film

I. GENERAL	309
II. PHYSICAL PROPERTIES	310
III. GENERAL REMARKS ON CHEMICAL COMPOSITION	312
IV. FATTY ACIDS	313
A. Free and Combined Acids	313
B. The Fatty-Acid Series of Wool Fat	314
C. The Fatty-Acid Series of Human Hair Fat	317
D. The Biological Significance of the Occurrence of Fatty Acids on the Surface	318
V. ALCOHOLS	321
A. Wax Alcohols	321
B. Sterols	322
1. Sterols in General	322
2. Cholesterol	324
C. Glycerol	325
D. Triterpenoid Alcohols	326
VI. HYDROCARBONS	328
A. Squalene	328
B. Other Hydrocarbons	329
VII. VITAMINS AND VITAMIN PRECURSORS	329
A. Pro-vitamin D	329
B. Vitamin A and Precursors	330
C. Vitamin E	330
D. Other Vitamins	330
VIII. HISTOCHEMICAL OBSERVATIONS	330

I. GENERAL

THE skin surface and hair of mammals are covered with a half-aqueous, half-greasy film. The film appears to be homogeneous, because the fatty and aqueous phases form a fine emulsion, the main emulsifiers being cholesterol and wax alcohols. Considerable biological significance is attributed to this film. It is supposed to protect the skin

against the vicissitudes of the atmosphere, such as overwetting, overdrying, and abrupt changes of temperature (p. 258); it slows down the absorption of foreign substances from the outside (p. 30); it protects against some exogenous infections and infestations by virtue of some of its chemical constituents (p. 318); it carries—as evidenced by

biological, but not chemical, data—the precursor of vitamin D (p. 329), which is transformed into vitamin D by ultraviolet radiation of the sun; and, finally, it contains species- and sex-specific odorous substances involved in sexual attraction and in recognition of the own and foreign species (113).

The main source of lipid material in this film are the sebaceous glands. The second source is the keratinizing epidermis: the invisible horny lamellae which are steadily cast off carry a great amount of lipid substances. Apocrine glands also excrete lipid droplets. Whether the eccrine glands contribute to the maintenance of the fatty film is rather doubtful.

By definition, the term “sebum” should be reserved to designate the product of sebaceous glands only. In birds it is relatively easy to obtain the pure product of their sebaceous preen glands (p. 285). In mammals, however, it is extremely difficult to separate the sebum from the lipids deriving from the horny layer. A remarkable attempt in this direction was made by Schmidt-Nielsen, Suskind, and Taylor (114). Conclusions on the composition of pure human sebum were drawn on the basis of data derived from the analysis of dermoid and sebaceous cyst materials. However, the content of such cysts differs from the sebum freely expelled to the surface; for instance, they do not contain free fatty acids. Moreover, one cannot be

sure whether these cysts do or do not contain lipid products of keratinization.

One way to study pure sebaceous-gland excretion products in man would be to collect the lipids from palms and soles, which have no sebaceous glands, and to compare the composition of this fat with the surface lipids from other regions. In this way specific products of the sebaceous glands may be detected which do not occur on palms and soles but are found elsewhere. Such differential analysis was attempted by Unna and Golodetz (126, 127). Apparently, it is very difficult to keep palms and soles free from contamination by the easily flowing sebum of dorsal parts (52), but it does not seem to be impossible (45). On the other hand, any conclusions from such differential data would imply that the products of keratinization on palms and soles are the same as elsewhere, a contention which has not been proved.

It is generally assumed that if the surface fat has been thoroughly removed with fat solvents, the sebaceous glands start immediately to produce and excrete their product in considerable amounts, while the replenishment of horny fat is a rather slow process (30, 31). The validity of this assumption may be questioned. But there is some probability that if skin surface fat is collected early—within 1–2 hours—after thorough defatting, a relatively great part of this fat stems from sebaceous glands (52).

II. PHYSICAL PROPERTIES

Sebum is liquid inside the duct and hair follicle. It diffuses up and down in the follicular canal, and after reaching the surface it diffuses all over the skin and also penetrates between the horny lamellae of the stratum disjunctum. Butcher and Parnell (11) found that on the surface it spreads primarily along the sulci between the follicular orifices.¹ It solidifies at around skin surface temperature (at about 30° C.) and

normally rests on the skin and hair in a semi-solid state, like a very thin layer of a hydrophilic ointment. Sebum has no sharp freezing point because of the heterogeneity of the fatty mixture (9), but it becomes quite hard below 20° C. Butcher and Coonin (9) found a critical change in viscosity of human surface fats between 37° and 28° C., with a most abrupt change from 30° to 28.5° C. (Fig. 1). Such an abrupt change cannot be recognized from the curves of Lincke (66) (Fig. 2). Still the great change in viscosity in the range of the physiologically occurring tem-

1. Sulzberger and Herrmann found that sweat droplets spread in the same way (personal communication).

peratures is clearly shown in both sets of data and obviously is of biological significance (p. 289).

With "freezing" of the sebum, there is separation of liquid and solid phases (9). Apparently, such separation does not occur on the skin surface because of the presence of water and the emulsified state of the lipids. Similarly, if surface fat is handled at 15°–17° C., it ceases to flow (9), a situation which might be quite different in the presence of water. In this connection it might be interesting to note that sebaceous material as expressed from individual pores contains from 18 to 27 per cent water (114).

The specific gravity of skin surface fat, collected from the forehead and measured at 20° C., was found to be 0.911 (9); collected from the trunk, 0.9286 (66). The surface tension of lipids from the trunk measured by

the capillary ascension method was 29 dynes/cm, and measured stalagmometrically, 30.5–31.1 dynes/cm at 18° C. (66). Butch-

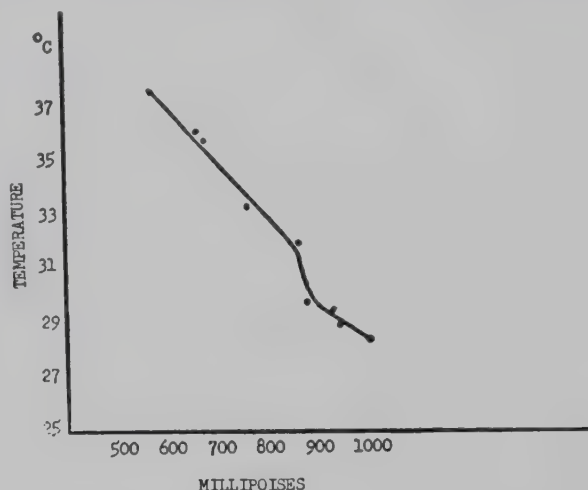


FIG. 1.—Effect of temperature on the viscosity of human sebum. From Butcher and Coonin (9). (Reproduced by permission of the Williams & Wilkins Company.)

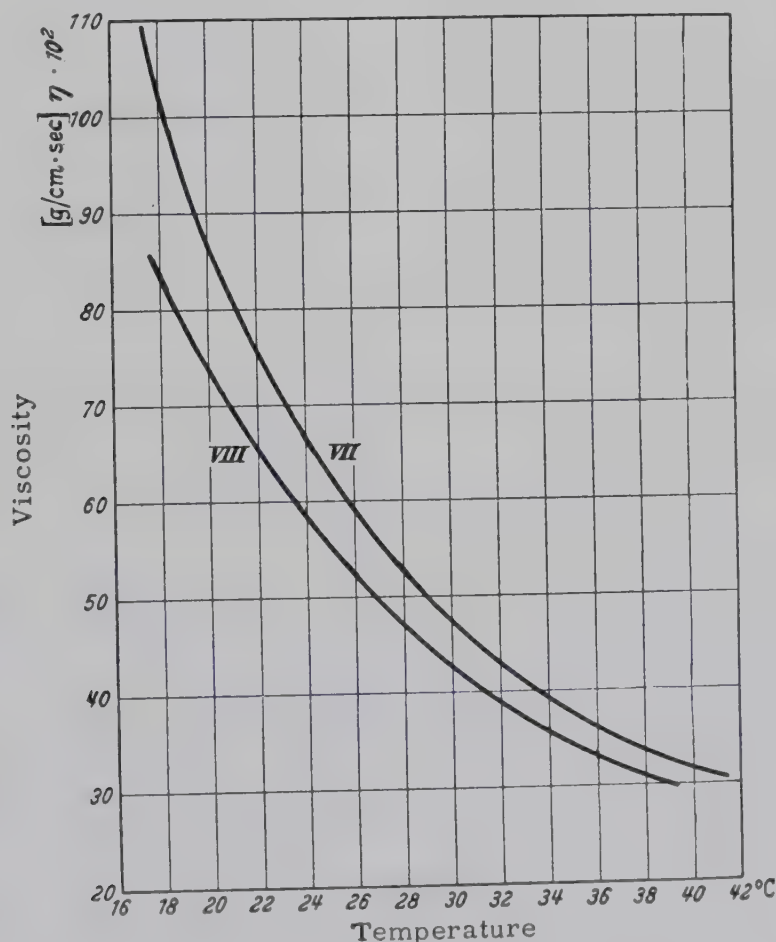


FIG. 2.—Effect of temperature on the viscosity of human sebum. From Lincke (66). (Reproduced by permission of Springer-Verlag.)

er and Coonin (9) carried out measurements on forehead fat with the capillary ascension method between 26.5 and 31° C. Their average value was 24.89 dynes/cm.

Biologically, the miscibility with water and the "freezing" at low temperatures are the most important features of skin surface fats.

III. GENERAL REMARKS ON CHEMICAL COMPOSITION

Chemically, skin surface fats are vastly different from the fat of the subcutaneous tissue and of other fat depots inside the body. These depots consist, in the main, of triglycerides of palmitic, stearic, and oleic acids, with the admixture of only small amounts of other lipid materials. Surface fats are much more complex. In man, skin surface fats contain free (nonesterified) fatty acids and fatty acids esterified with cholesterol, with wax alcohols, and with glycerol. They also contain free cholesterol and hydrocarbons, one of the important hydrocarbons being squalene. The wool fat of sheep contains triterpenoid alcohols instead of squalene. It does not contain glycerol at all (26). Somewhat schematically, one may enumerate the characteristics of surface fats as follows:

1. Free fatty acids are present. These acids are members of homologous series ranging from short to very long chains.
2. The same series of fatty acids is obtained from the esterified portion of skin fats.
3. Wax alcohols and hydrocarbons are also represented in homologous series.
4. There are relatively few glycerides compared to depot fats, and the unsaponifiable portion is large.
5. Only traces of nitrogen and phosphorus are present.
6. The fat mixture is hydrophilic. The hydrophilia is greater than if it were due to the presence of cholesterol only.

Microanalytical data on the average composition of human surface fats were published by Zehender (146) and by Kvorning (58). Analyses on a macroscale were recently performed by MacKenna, Wheatley, and Wormall (73, 134, 74) on surface fats of the forearm of adult males, and by

Nicolaides and Rothman (89) on hair fat of males, females, and children.

The quantitatively most variable constituents are the free fatty acids. The high values of Nicolaides (89), shown in Table 1, are probably due to lipolytic decomposition of fatty-acid esters during storage of hair (p. 313). The free fatty-acid content of relatively fresh material varies from 20 to 40 per cent of the total fat, according to most data in the literature.² There is general agreement on the high content in unsaponifiable material (p. 322), the average being around 30 per cent in humans and up to 50 per cent in sheep (26).

Data used in fat chemistry for characterizing fat mixtures, such as acid number, iodine number, saponification number, etc., were often estimated in different surface fats and their fractions. They will not be reviewed here, partly because they were obtained from highly variable mixtures or from not always well-defined fractions and partly because since the estimations of those data important steps have been made toward the identification of homologous series and single compounds in surface fats. It is hoped that actual detailed chemical analysis will soon make it superfluous to manipulate with ill-defined fractions.

As in other areas of biochemistry of the skin, e.g., the structure and physicochemical properties of keratins, collagen, etc., industrial chemistry took the lead in the study of skin surface fats also, probably because of the commercial significance of wool fat. Up to recent times, hardly any work had been done in this field by workers with primarily

2. In recent analyses of very fresh material Nicolaides found extremely low free fatty-acid values. Apparently, the great bulk of free fatty acids is formed by hydrolysis after expulsion of the material to the surface (unpublished).

biological and medical interest. In the last ten years, however, this situation has changed. Human skin surface fats have been studied, and the emphasis has shifted from

being manufactured and how these biochemical processes change under different physiological and pathological conditions in the living organism.

TABLE 1
GROSS COMPOSITION OF HUMAN HAIR FAT (89)

Human Origin of Fat	How Fat Obtained	Pooled Samples Analyzed	Per Cent Free Fatty Acids	Per Cent Esterified Acids	Per Cent Total Acids	Per Cent Unsaponifiable	Per Cent Recovery
White boys, 6-12.....	Cut hair extract	3	44.6	16.2	60.8±1.0	36.6±1.3	97.4
White men.....	Cut hair extract	4	54.6	14.0	68.6±1.8	30.0±1.8	98.6
White men over 65...	Cut hair extract	1	57.2	12.3	69.5	28.6	98.1
Negro men.....	Cut hair extract	2	57.2	13.9	71.1±0.5	27.4±0.2	98.5
White women.....	Cut hair extract	3	63.4	15.3	78.7±0.6	21.3±1.2	99.7
White women over 65.	Cut hair extract	1	57.8	18.1	75.9	22.2	98.1
Negro women.....	Cut hair extract	1	62.8	11.8	74.6	23.4	98.0
White man "P".....	Scalp wipings	1	35.6	35.3	70.9	27.4	98.3
White man "A".....	Scalp wipings	1	39.8	30.6	70.4	28.2	98.6
White man "L".....	Scalp wipings	1	41.2	27.2	68.4	29.8	98.2
White man "S".....	Scalp wipings	1	33.1	32.6	65.7	32.4	98.1
Young men*.....	Forearm†	30.0	37.5	67.5	32.5

* An average of data of MacKenna, Wheatley, and Wormall (73, 74).

† Chloroform extract of fat obtained from immersion of forearm in acetone.

analytical and physical chemistry, useful from an industrial point of view, to the biochemical and medical problems of how and where the components of the lipid film are

Since most available data on skin are concerned with sheep and man, we shall deal mainly with these two species, pointing out some of the remarkable species differences.

IV. FATTY ACIDS

A. FREE AND COMBINED ACIDS

Fatty acids occur on the skin surface in the free state and esterified with glycerin, with wax alcohols, and with sterols. The relative percentage of free fatty acids in the total fatty mixture is variable. One reason for the variability probably is that fat deposited on the skin surface may undergo lipolysis under the influence of lipolytic enzymes derived from bacteria and fungi on the skin and in the atmosphere, and possibly of lipases of cutaneous origin (p. 331). Nicolaides (89) observed in my laboratory that if hair is stored, the free fatty-acid fraction of the hair fat increases with the length of storage time. Pooled skin surface fat of any source seems to have a higher free acid content than does fresh material from

single individuals (146). Also it was found that the acid number increases and the amount of esterified acids decreases with the time elapsing after a bath (12). Therefore, for estimation of the amount of free fatty acids, only freshly excreted material should be analyzed. There can be little doubt that skin surface fats do contain some free acids when they arrive at the surface. But it is not known whether they are primary products, i.e., acids which were never esterified, or whether they have been formed from esters by hydrolysis below the surface. They may well derive from hydrolytic decomposition of phospholipids (p. 489).

In wool fat the amount of free acids is about 11 per cent of the total weight of the fat (135). It seems to be considerably higher

in man. MacKenna *et al.* (73) obtained an average of 30 per cent from the surface fat of the forearm, and similar or higher values were obtained by Nicolaides (89) from the fat of freshly cut hair and from wipings of the scalp 24 hours after washing.³ Surprisingly low values (an average of 17 per cent) were obtained by Lincke (66), who collected material by extracting underwear.

If the material is fresh and taken under controlled conditions with a standardized time elapsing after the last defatting, standardized procedure of fat removal, standardized area, etc., it seems that the per-

"neutral fat portion," can be saponified, whereby the esterified acids are liberated. The total amount of these combined acids is 44 per cent in wool fat (135). In man the following data were obtained: 30–40 per cent (32), 34–57 per cent (146), average 36 per cent (73), average 32.5 per cent (134), average 38 per cent (89). It appears that there is an inverse relationship between the percentages of free and esterified acids: where the amount of free acids is high, the amount of esterified acids is low, and vice versa (Table 1).

From the biological point of view it is important that the same acids occur in both the free and the esterified state. Free and bound acids belong to the same homologous series and have approximately the same relative distribution of the single members within the series (Table 2). It might be assumed, therefore, that in the process of sebum formation there is either, first, a synthesis of free fatty acids which undergo partial esterification, with some of the fatty acids remaining free; or, instead, all fatty acids become esterified and part of them are hydrolyzed into free acids and alcohols.

TABLE 2*

COMPARISON OF FREE AND ESTERIFIED ACID FRACTIONS

Acid	Fatty Acids from Neutral Fraction (Per Cent)	Free Fatty-Acid Fraction (Per Cent)
Below C ₁₄ . . .	2.7	5.8
C ₁₄ . . .	7.2	9.5
C ₁₅ . . .	7.5	6.0
C ₁₆ . . .	31.8	36.0
C ₁₇ . . .	6.6	6.0
C ₁₈ . . .	26.5	23.0
Above C ₁₈ . . .	17.0	14.5

* Weitkamp, Smiljanic, and Rothman, unpublished.

centage of free fatty acids and the acid number are fairly constant in the same individual (146, 41). More or less characteristic individual differences seem to exist (146, 41). If the influence of any factor on the excretion of free fatty acid is to be examined, it should be done on the same individual or the same group of individuals in pre-experimental and experimental periods.

After removing the free fatty acids, the remaining fat mixture, usually called the

3. In my publication on the fungistatic action of free fatty acids on the scalp (106), only 6 per cent free fatty acids were reported. It was thought (58, 59) that these values are unreliable because the hair was collected from barber-shop sweepings. Actually, in that work we did not attempt an exhaustive extraction of free fatty acids, being interested only in the biological properties of the free acid fraction but not in its quantity.

B. THE FATTY-ACID SERIES OF WOOL FAT

The old literature on the fatty-acid series of wool fat has often been reviewed (104, 130, 38), more recently in great detail by Truter (124). Older data on the isolation and identification of single compounds became obsolete when Weitkamp (135) in 1945 demonstrated that there are four homologous series of fatty acids in wool fat (Table 3).

The first series comprises nine straight-chain saturated acids with only even-numbered carbon atoms from C₁₀ to C₂₆, viz., capric (decanoic), lauric (dodecanoic), myristic (tetradecanoic), palmitic (hexadecanoic), stearic (octadecanoic), arachidic (eicosanoic), behenic (docosanoic), tetracosanoic, and cerotic (hexacosanoic). Several of these acids were isolated from wool fat before Weitkamp's work, such as myristic, palmitic, stearic, and cerotic (104, 26). Drum-

mond and Baker (26) found cerotic particularly richly represented.

The second series of acids found by Weitkamp comprises two 2-hydroxy acids, one with 14 and one with 16 carbon atoms. The latter compound is identical with the lanopalmitic acid ($C_{16}H_{32}O_3$) first described by Darmstädter and Lifschütz (17) and recovered from wool fat also by Kuwata and Ishii (57). The presence of a "lanoceric" hydroxy acid (C_{30} or C_{32}) was also confirmed (57, 71). According to Truter (124), these hydroxy acids probably

fied in vertebrates. However, the C_{20} member of this series had previously been isolated from wool fat by Abraham and Hilditch (1).

The fourth group represents the largest number of members, namely, twelve, all having their branching methyl group on the second next to the last carbon atom (anteiso series). All the members have odd-numbered carbon atoms, from C_9 to C_{31} .

These novel findings of Weitkamp were important steps toward a better understanding of the biochemical processes taking

TABLE 3
FATTY ACIDS ISOLATED FROM WOOL FAT (135)

Homologous Series	General Formula	<i>n</i>	No. of C Atoms (Even or Odd)	Isolated Members of Series
1. Normal fatty acids.	$CH_3-(CH_2)_{2n}-COOH$	4-12	Even	$C_{10}-C_{26}$
2. 2-hydroxy acids.	$CH_3-(CH_2)_{2n-1}-\underset{\substack{ \\ OH}}{CH}-COOH$	6-7	Even	$C_{14}-C_{16}$
3. Isoacids with methyl side chain in penultimate position	$CH_3-\underset{\substack{ \\ CH_3}}{CH}-(CH_2)_{2n}-COOH$	2-11	Even	$C_{10}-C_{28}$
4. Anteisoacids with methyl side chain in antepenultimate position.	$CH_3-CH_2-\underset{\substack{ \\ CH_3}}{CH}-(CH_2)_{2n}-COOH$		Odd	C_9-C_{31}

form diesters in wool fat, the $-OH$ group being linked with an acid and the $-COOH$ group with an alcohol. In a more recent analysis Horn, Hougen, and Von Rudloff (48) found that nearly 30 per cent of the fatty acids are hydroxylated. The normal α -hydroxy acid derivatives of lauric (C_{12}), myristic (C_{14}), palmitic (C_{16}), and stearic (C_{18}) were isolated and quantitatively estimated.

The third series comprises ten branched-chain acids with even numbers of carbon atoms between C_{10} and C_{28} . The branching methyl group in this series branches off from the carbon, which is next to the last in the chain away from the carboxyl group. Such series of isoacids were not previously identi-

place in sebaceous glands. The following points are particularly noteworthy:

1. It became clear that single compounds are not synthesized in a random fashion but that complete homologous series are built by the glands. It is true that some members are present in much larger amounts than others, indicating a greater ease of manufacturing certain chain lengths, for instance, C_{14} and C_{16} in the first and second series. But, nevertheless, the homologous series are complete throughout. Abraham and Hilditch (1) were inclined to assume that there is a certain preference for chains with 5 carbons or multiples thereof (particularly C_{15} , C_{20} , C_{30}) and hypothesized that this is because the acids are built of isoprene fragments (p.

326). This hypothesis had to be abandoned in the light of Weitkamp's findings.

2. For the sheep's skin it is easier to build even-numbered carbon chains with some carbon skeletons and odd-numbered carbon chains with other skeletons.

3. All members of the fourth (anteiso) series have odd numbers of carbon atoms. Prior to these findings it was generally assumed that chains with odd numbers of carbon atoms do not occur in natural fats, except the short C_1 , C_3 , and C_5 chains. The explanation for nature's preference for the production of even-numbered chains has been that, in tissue catabolism, carbon chains are decomposed into 2-carbon fragments and that in the anabolic phase the new compounds are built up from these 2-carbon breakdown products. This concept has been greatly supported by experiments with radioactive isotopes. From Weitkamp's work it appears that skin surface fats constitute an important exception to the rule of the exclusive formation of even-numbered carbon chains. So far, skin fats are the only fatty products in the mammalian body containing a considerable proportion of compounds with odd numbers of carbon atoms. How this is achieved is obscure. Earlier assumptions that synthesis may start from 3- or 5-carbon fragments cannot be applied to Weitkamp's C_{11} , C_{13} , C_{19} , C_{21} , C_{23} , and C_{31} chains. One may assume, however, that sebaceous glands possess the (enzymatic?) ability to oxidize the α -carbon atom of even acids, with subsequent decarboxylation. The other possibility is that very long even-numbered carbon chains are first built and that then they are broken into equal or unequal fragments with odd-numbered carbon atoms (p. 322).

4. The increase in the chain length to 31-carbon atoms in the anteiso series is again a unique feature. Chains longer than C_{18} do not occur or occur only in traces in other animal fats. These long chains illustrate best the point that sebum is not simply formed by uptake of fat from the blood stream but is the result of a highly specific synthetic process (104).

5. The shape of branching of the carbon skeleton in the iso series corresponds to that of valine,



and in the anteiso series to that of isoleucine,



Possibly the synthesis of the iso- and anteiso-acids starts from these amino acids after deamination.

6. The branched-chain homologous series of acids are species-specific. Or, at least, so far they have been shown to occur only in sheep. They certainly are not present in human skin surface fats (see below). From the preen glands of ducks some branched-chain fatty acids were isolated (137); but, while the branching of the C_7 acid corresponded with that of the anteiso series, the next member could not be identified as such. Also a branching C_{18} acid was found to be present in preen-gland oil (137), but the position of the branch could not be established.

7. Branched-chain fatty acids were known to occur in bacteria, such as the tubercle bacillus (3), *Pseudomonas tumefaciens* (128, 129), and, interestingly, also in microorganisms with antibiotic potency, such as the polymyxins (13). Their occurrence in the mammalian organism has been a novel finding.

As Truter (124) pointed out, Weitkamp did not deal with the unsaturated acids of wool fat. Truter found that about 15 per cent of the total acids are unsaturated. It has often been claimed that fatty acids of low molecular weight occur in wool fat. The presence of the monobasic acids, butyric, isovaleric, and caproic (18); of the dibasic acids, succinic and glutaric (90); and of the hydroxy acid, lactic (36), was reported. Whether these acids derive from sebaceous glands, from horny material, or from apocrine or eccrine sweat glands is not known. Lactic acid is a characteristic product of ec-

crine glands (p. 210) but not of the others. In spite of the great biological significance of these compounds, little attention has been paid to them in modern biochemical literature. (Concerning citric acid see p. 475.)

C. THE FATTY-ACID SERIES OF HUMAN HAIR FAT

There is only one homologous fatty-acid series in human hair fat, a series of normal, straight-chain monobasic fatty acids (136). With the exception of C_{19} and C_{21} , all members with even and odd numbers of carbon atoms from C_7 to C_{22} were isolated and identified after fractional amplified distillation of their methyl esters (136). Again, as in wool fat, the chain lengths C_{14} , C_{16} , and C_{18} are predominant (Table 4), but the others are also present in measurable amounts, and some of the odd-numbered members, such as the C_{15} and C_{17} acids, contribute a considerable fraction to the total. Odd-numbered normal acids have not been shown previously to be synthesized in nature. They are highly specific products of human sebaceous glands.

Both saturated and unsaturated acids of this series were isolated. No unsaturated

in the lower members can possibly be explained by the peculiar position of the double bond in the higher members (Fig. 3). In the unsaturated compounds most of the

TABLE 4

APPROXIMATE COMPOSITION OF METHYL ESTERS OF FREE FATTY ACIDS OF HUMAN HAIR FAT (136)

Carbon Atoms	Per Cent Present	Per Cent Unsaturated
7.....	0.07	0
8.....	0.15	0
9.....	0.20	0
10.....	0.33	0
11.....	0.15	Trace
12.....	3.5	4 (-2H)
13.....	1.4	3 (-2H)
14.....	9.5	15 (-2H)
15.....	6.0	25 (-2H)
16.....	36.0	50 (-2H)
17.....	6.0	67 (-2H)
18.....	23.0	80 (-2.4H)
20.....	8.5	85 (-2.5H)
22.....	2.0
Bottoms	4.0

double bonds were situated between the ninth and tenth carbon atoms as counted from the last carbon which is farthest from

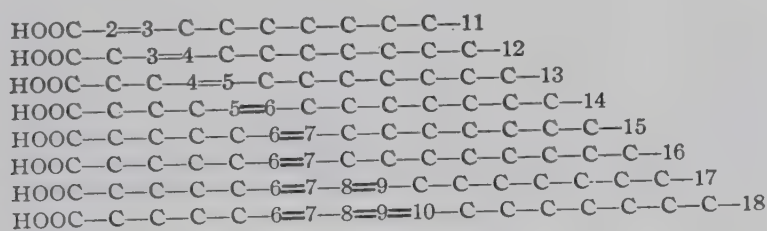


FIG. 3.—Position of double bonds in higher members of the unsaturated fatty-acid series of human hair fat. From Weitkamp, Smiljanic, and Rothman (136).

acids were present in the chain lengths C_7 to C_{10} , traces were present in the C_{11} fraction, and, from then on, the relative amount of unsaturated compounds rapidly increased with increasing length of the chain (Table 4). Only 15 per cent of the C_{14} acids are unsaturated; but at the C_{20} level the proportions are already reversed: 85 per cent are unsaturated and only 15 per cent saturated.

The absence of unsaturated compounds

the carboxylic carbon. If such an acid is split at the double bond, one fragment will always be of the length of 9 carbon atoms. Thus, apparently, double-bond formation by sebaceous glands is possible only at chain lengths longer than C_{10} .

In addition to monoethenoid acids, di- and triethenoid compounds (compounds with two and three double bonds) also were isolated. The C_{18} fraction yielded the pro-

portions shown in the accompanying tabulation.

	Per Cent		Per Cent
Oleic.....	80.8	Linolenic...	1.8
Linoleic.....	14.8	Stearic.....	2.6

It is not known whether those isomers of linoleic and linolenic acids are synthesized which are known to be essential, not being synthesized anywhere else in the body (p. 484). Possibly the observation of Smedley-MacLean and Hume (118) that rats fed fat-free diets for a long time have a much higher lipid content in their skins than in other organs may be linked to the production of these fatty acids by sebaceous glands. Once these essential fatty acids are present, fat can be synthesized from carbohydrates, but not otherwise (6) (p. 684).

D. THE BIOLOGICAL SIGNIFICANCE OF THE OCCURRENCE OF FATTY ACIDS ON THE SURFACE

Peck (92) was the first to suspect that the presence of low-molecular free fatty acids on the surface of human skin may play a role in hindering the growth of pathogenic organisms. His work has led to the highly successful development of fatty-acid treatment of superficial fungus infections of the skin.⁴ Peck thought that the free fatty acids are sweat-gland products because he was unable to demonstrate any fungistatic action of sebum taken from sebaceous cysts. Actually, it has been shown since that no free fatty acids are present in the sebum produced in cysts (134), if there is no fermentation; and obviously this is the reason for the inactivity of such material.

It is highly improbable that free fatty acids are products of eccrine-gland function.

4. See, e.g., M. B. Sulzberger, H. C. Shaw, and A. Kanof, Evaluation of measures for use against common fungous infections of the skin, U.S. Nav. M. Bull., 45:237-48, 1945; A. L. Shapiro and S. Rothman, Undecylenic acid in the treatment of dermatomycosis, Arch. Dermat. & Syph., 52: 166-71, 1945; J. G. Hopkins, J. K. Fisher, A. B. Hillegas, R. B. Ledin, C. G. Rebell, and E. Camp, Fungistatic agents for treatment of dermatophytosis, J. Invest. Dermat., 7: 239-53, 1946.

Chemically no such acids were isolated from profusely flowing sweat, and Peck himself obtained very modest fungistatic action from highly concentrated samples of sweat. It was pointed out (86) that the most common fungus infection in man, tinea pedis, or "athlete's foot," is located exactly in those areas where sebaceous glands are lacking but where eccrine sweat is abundant. This particular infection does not spread beyond the nonsebaceous areas, because the causative fungi are too sensitive to the free fatty acids produced by sebum. Certainly, two pathogenic species of fungi, *Trichophyton mentagrophytes* and *T. purpureum*, preferentially colonize in areas which are not supplied by sebaceous glands. However, they may acquire a limited resistance to fatty acids (86) and then spread to sebum-covered areas (102).

A valuable contribution to the biological role of fatty acids was made by Burtenshaw (7), who in painstaking experiments demonstrated that free fatty acids on the skin contribute to the so-called "self-sterilization" of the skin. Reviews on this subject were published by Burtenshaw in 1945 and 1948 (8). This self-sterilization is a rather incomplete one, since only certain microorganisms are inhibited on human skin while others are not. Furthermore, the Pillsbury group (95, 94) has shown that drying is one of the most important factors in the disappearance of bacteria from the skin. Nevertheless, the data on this limited bacteriostatic action of free fatty acids, as demonstrated by Burtenshaw, have remained valid. In an extensive study Ricketts, Squire, and Topley (98) found that both drying and the presence of unsaturated fatty acids play a role in the disappearance of bacteria from the skin surface: for *Streptococcus pyogenes* the major factor is the action of unsaturated fatty acids; for *Pseudomonas pyocyanea* and *Bacterium coli* the major factor is drying; for *Staphylococcus aureus* both unsaturated fatty acids and drying have a demonstrable effect.

Microsporon audouini, the most common causative agent of ringworm of the scalp in children, is about ten times more sensitive

to the fungistatic action of fatty acids than are other filamentous pathogenic fungi (97, 96). This infection clears spontaneously during puberty, and the scalp of adults is immune to it. Rothman *et al.* (105, 107, 106), studying the fungistatic action of hair fat and its fractions, came to the conclusion

is unable to penetrate into the hair shaft. However, by entering the follicular canal and the horny layer, it prevents infection of the new hair which follows the old hair in the process of shedding.

This work was challenged by Kligman (54) on the basis that he was unable to du-

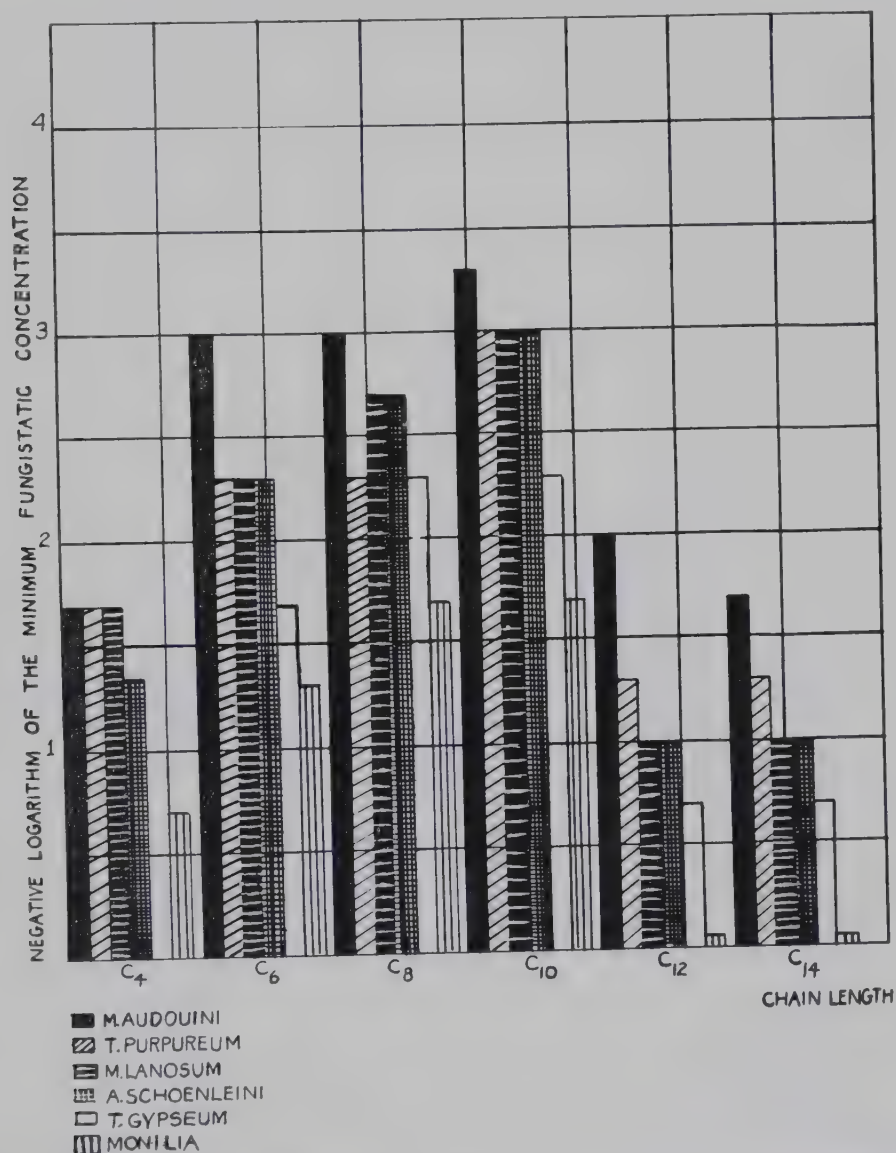


FIG. 4.—Minimum fungistatic action of the C₄ to C₁₄ even-numbered normal saturated fatty acids on six pathogenic fungi. (E. L. Laden, unpublished.)

that the cure in puberty and the immunity of the scalp of adults are due to the pubertal development of sebaceous glands, whereby higher concentrations of the fungistatic fatty acids are expelled to the surface. The "adult type" of hair fat, they found, does not kill the fungi within the hair because it

plicate experimental results of Rothman *et al.* on the gram per gram higher fungistatic action of adult hair fat as compared with that of children's hair fat. He justly criticized the methods available for quantitative measurements of fungistatic action. However, it was pointed out (101) that, even if

Kligman's results were conclusive, the great (about threefold) increase in the amount of sebum during puberty (p. 296), as Kligman himself demonstrated, should amply suffice to raise the free fatty-acid concentration per surface area beyond the critical

which is caused by the most fatty-acid-sensitive fungus (97, 96).

Rothman *et al.* (106) found that the odd-numbered saturated fatty acids—C₇, C₉, C₁₁, and C₁₃—all occurring in human hair fat, are more potent fungistatic agents than

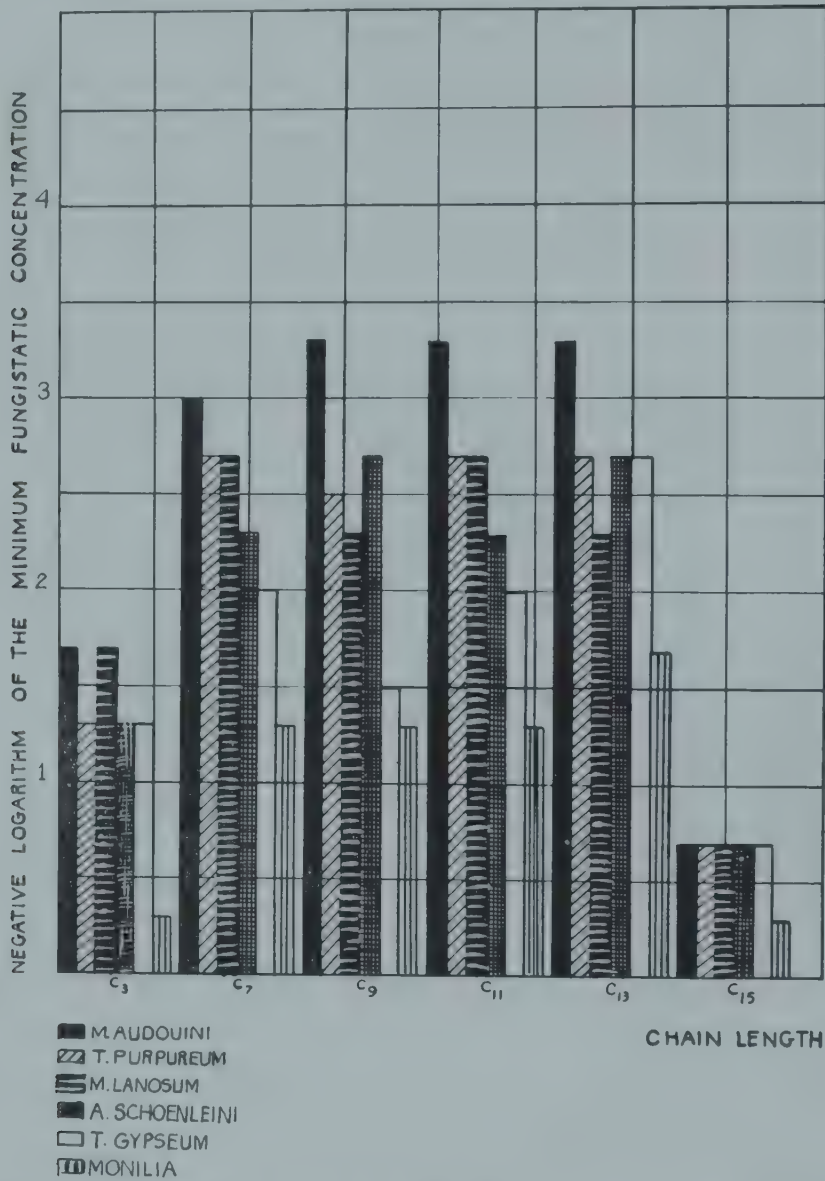


FIG. 5.—Minimum fungistatic action of the C₃ to C₁₅ odd-numbered normal saturated fatty acids on six pathogenic fungi. (E. L. Laden, unpublished.)

level at which *Microsporon audouini* is no longer able to thrive. It is rather suggestive that, among all dermatophyte infections, only *Microsporon audouini* infection of the scalp is known to disappear spontaneously during puberty and that it is this infection

their even-numbered neighbors. E. L. Laden, in my laboratory, studied the minimum fungistatic concentration of normal saturated fatty acids with even and odd numbers of carbon atoms. His unpublished results are shown in Figures 4 and 5.

V. ALCOHOLS

A. WAX ALCOHOLS

Waxes are esters of long-chain fatty acids and long-chain aliphatic alcohols. A representative example is ceryl palmitate, an ester of the straight-chain alcohol ceryl alcohol, $C_{26}H_{53}OH$, and palmitic acid. Ceryl palmitate is the main constituent of beeswax. Such waxes are not found in vertebrate metabolism except in skin excretions.

The first wax alcohol to be isolated from skin surface fats was cetyl alcohol, $C_{16}H_{33}OH$, from sperm-whale oil. It occurs there mainly in the form of its palmitate ester (35). Later followed the isolation of octadecyl alcohol, $C_{18}H_{37}OH$, from the preen-gland excretion of water birds (99) (48 per cent of preen-gland lipids is octadecyl alcohol [137]); of eicosyl alcohol, $C_{20}H_{41}OH$, from dermoid fat of women and tentatively from human sebum (70); and of ceryl alcohol, $C_{26}H_{53}OH$, from wool fat (17). I have summarized the older data (104). Newer reviews have been published by Velluz and Lederer (130) and Truter (124).

In wool fat the presence of the C_{18} , C_{20} , C_{22} , C_{24} , and C_{26} normal (straight-chain) saturated alcohols was recently demonstrated by Tiedt and Truter (123). In addition, data are accumulating which show that not all wax alcohols in wool fat are straight-chain and monovalent alcohols. Kuwata and Katuno⁵ found an octadecyl alcohol with a melting point different from that of normal octadecyl alcohol. They also isolated a glycol (an alcohol with two hydroxyl groups) with the tentative structural formula $C_{21}H_{40}(OH)_2$, which they called "lanyl alcohol." Horn and Hougen (47) reported on the presence in wool fat of a series of dihydroxy alcohols with chain lengths C_{16} , C_{18} , C_{20} , C_{22} , and C_{24} . All these alcohols were 1:2-diols. Indirect evidence suggested that they have branched chains, probably a single methyl branch, as happens also in the iso and ante-

iso series of acids (p. 315). This has been confirmed recently by Murray and Schoenfeld (87), who isolated ten branched-chain alcohols: six anteisoalcohols of odd carbon number, C_{17} – C_{27} , and four isoalcohols of even carbon number, C_{20} – C_{26} . The great similarity in structure of acids and alcohols in wool fat is certainly striking. Recently, Von Rudloff (109) succeeded in separating monovalent and bivalent, straight-chain and branched-chain, alcohols of wool fat by combining chromatographic methods with the urea-separation method.

On human sebum little work has been done as yet toward isolation of single wax alcohols. From human dermoid cysts Dimter (19) isolated six of the saturated monovalent normal alcohols, all with even-numbered carbon atoms, viz., myristyl, C_{14} ; cetyl, C_{16} ; octadecyl, C_{18} ; eicosyl, C_{20} ; docosyl, C_{22} ; and carnaubyl, C_{24} , alcohols. MacKenna, Wheatley, and Wormall (74) found a saturated alcoholic substance in the unsaponifiable fat of the forearm skin surface which appeared to them to be eicosyl alcohol, though not characterized to their complete satisfaction. In our laboratory Nicolaides (89) separated the wax alcohols of human hair fat chromatographically. His fraction consisted chiefly of straight-chain compounds as determined by urea-adduct formation. There were saturated as well as unsaturated compounds, the relation being about 4 to 1. The alcohols formed a homologous series ranging from C_{16} to C_{26} , as determined by mass spectrometry. Odd- as well as even-numbered compounds were present, the former in much smaller amounts. These alcohols were all primary alcohols, as determined by infrared spectroscopy. The average molecular weight was 294, corresponding to an average chain length of about C_{20} . Children's hair fat had about half as much wax alcohols as did that of either men or women.

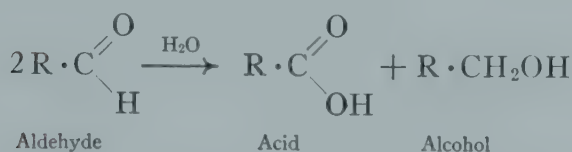
The available data on wax alcohols in skin surface fats, though scanty, permit one

5. T. Kuwata and M. Katuno, Wool wax. IV. Two new alcohols in wool wax, *J. Soc. Chem. Indust. Japan*, **41**:227–28, 1938. Quoted in *Chem. Abstr.*, **32**:9536, 1938.

conclusion, namely, that the series of alcohols closely corresponds to that of acids. In wool fat only even-numbered normal acids and only even-numbered normal alcohols were found, and the branched-chain iso- and anteisoacids correspond to similar alcohols. In human surface fat the whole series of straight-chain and odd-numbered normal acids are present, and so are the normal alcohols. Even the prevalence of single members in the alcohol and in the acid series seems to match fairly well.

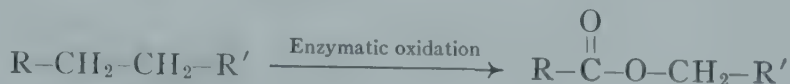
Of course, this matching is not perfect. In Nicolaides' human hair fat the average molecular weight of the crude alcohol portion was 294, while the molecular weight of acids calculated from neutral equivalents average about 270. This indicated about two more carbons for the alcohols, a difference confirmed by mass spectrographic analysis.

In the past it was assumed that acids and alcohols are built simultaneously from the same carbon skeleton, so that an aldehyde is formed first (91, 104), and then acid and alcohol are formed from the aldehyde by the Cannizzaro reaction, which is effected by the enzyme aldehyde-mutase:



If this mechanism operates at all, it cannot be the sole mechanism. It would imply equimolecular quantities of acids and alcohols, and this is obviously not the case.

Since the discovery of hydrocarbon chains as long as C₄₀ in human hair fat (p. 329), a new possibility is that these long-chain hydrocarbons are formed first from fragments with an even number of carbon atoms. These could possibly then oxidize at the centers directly to esters,



with *R* and *R'* being either odd or even aliphatic chains.⁶

In what proportion the wax alcohols occur in esterified and in free form in the skin

surface fats is not yet finally established. Drummond and Baker (26) found that in wool fat only a small proportion is present in the free state. The recent work of Nicolaides (unpublished) strongly suggests that in human sebum all wax alcohols are esterified. He was unable to demonstrate the presence of any free wax alcohols in hair fat. He finds that apparently only glycerides are split when hair fat is stored. Fatty acids are not liberated either from waxes or from cholesterol esters.

The wax alcohols are listed throughout the literature as part of the "unsaponifiable" matter. In the light of the above findings, this expression might be misleading to the nonchemist. The unsaponifiable matter is defined as all the material which, after saponification of a fat mixture, can be taken up in fat solvents from the aqueous solution of soaps and glycerol (such as hydrocarbons, longer-chain alcohols, aldehydes, ketones, sterols). Wax alcohols and cholesterol go into the unsaponifiable portion, no matter whether originally they were esterified or not. Actually, waxes and cholesterol esters are saponifiable: they are split into alcohol and acid by the saponification procedure. Still these alcohols are parts of the unsaponifiable fraction. Clearly, the unsaponifiable fraction should not be taken as a measure of nonesteric unsaponifiable compounds but as a measure of fat-soluble material which remains after saponification and after removal of fatty acids.

B. STEROLS (35)

1. Sterols in General

Sterols are crystalline alcoholic substances found in the unsaponifiable matter of plants and animals. They consist of a saturated phenanthrene ring system, having

6. This possibility has become improbable since Nicolaides has found very much less straight-chained saturated hydrocarbon in freshly excreted hair fat (personal communication).

an additional five-membered ring fused to it. Most of the sterols contain 27–29 carbon atoms, with one secondary alcohol group. Some are completely saturated; others have one, two, or three double bonds. Cholesterol ($C_{27}H_{45}OH$) (Fig. 6) is the principal sterol of the animal organism and is found in all cells. Its ring system has an 8-carbon side chain attached to C_{17} , two angular methyl groups at C_{18} and C_{19} , one secondary alcohol group at C_3 , and one double bond between C_5 and C_6 .

In all tissues, cholesterol is accompanied by a saturated derivative—dihydrocholesterol or 3- β -cholestanol ($C_{27}H_{47}OH$). It dif-

ferently isolated by Nicolaides (unpublished). It constitutes about 1.2 per cent of the total sterol fraction.

The presence in wool fat of an “oxycholesterol” (Lifschütz, 64; older literature quoted in 104 and 66) with two alcoholic $-OH$ groups was not confirmed (80). There is a cholesterol oxide in wool fat which gives a strong Lifschütz reaction (115), but it is a 7-ketocholesterol and is not a natural product but probably arises from oxidation by air (16). The occurrence of an isomer of cholesterol, “metacholesterol” (Lifschütz, 64, 104), could not be confirmed (141, 142). Lederer *et al.* (16) found in wool fat two ad-

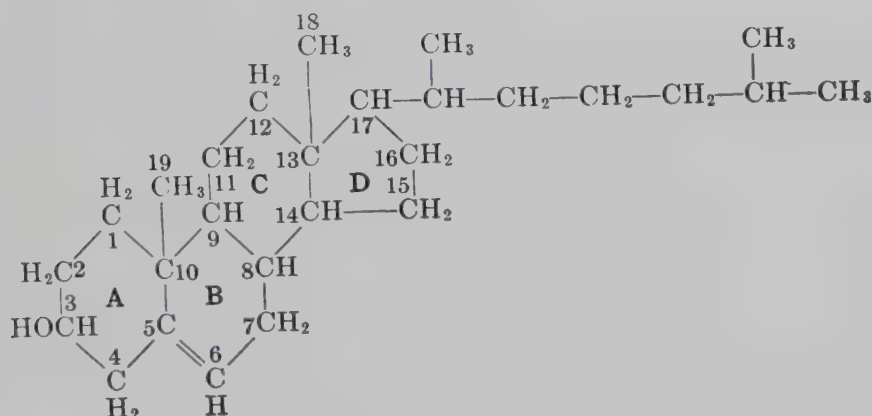


FIG. 6.—Cholesterol

fers from cholesterol only by the absence of the double bond. Like cholesterol, it is precipitated by digitonin; and, therefore, in all gravimetric estimations of cholesterol, which are based on this precipitation, the estimated amount of “cholesterol” has to be regarded as the total amount of cholesterol and dihydrocholesterol.

In wool fat the participation of dihydrocholesterol in the total “cholesterol fraction” is unusually high. Whereas in most tissues only 1–8 per cent of the sterol is present in the form of dihydrocholesterol, Schönheimer *et al.* (115) found in wool fat 18.9 per cent of the digitonin-precipitable material to be saturated sterols. Mohs (80) and Lederer *et al.* (16), however, reported only 9 and 8 per cent saturated sterols, respectively, in wool fat. In human skin surface fats the 3- β -dihydro derivative was

ditional cholesterol-ketones, one still unidentified but the other a 7-ketone with an additional double bond between 3 and 4, or, cholesta-3:5-dien-7-ketone.

Fieser (34) recently discovered a new sterol which accompanies cholesterol in many fats. This compound, Δ^7 -cholestenol, which he terms “lathosterol,” was found to be present to the extent of 2.97 per cent in wool fat. The same sterol was isolated from rat skin tissue by Baumann *et al.* (50) (p. 487). Besides this sterol, 7-dehydrocholesterol is present (140).

MacKenna *et al.* (74) found an uncharacterized sterol other than cholesterol in the unsaponifiable fraction of human skin fat from the forearm; this sterol was not precipitable with digitonin and was thought to be 7-hydroxycholesterol.

Nicolaides (unpublished) found in human

hair fat three sterols besides cholesterol. The first is dihydrocholesterol, mentioned before, and is found to accompany cholesterol in the ether eluate when the unsaponifiable portion is chromatographed on alumina. The second was found in the methanol eluate. It probably is 7-hydroxycholesterol and behaves in all respects like MacKenna's sterol. It is a "fast-acting" sterol (85), which means that it yields a rapidly developing color in the Liebermann-Burchard test. The third, also a fast-acting sterol, precedes cholesterol in the chromatogram of the unsaponifiable (benzene eluate) and is precipitable with digitonin. It is present in very small amounts.

2. Cholesterol

Cholesterol and its esters are present in all skin surface fats hitherto examined. It was noted early (70) that the cholesterol content of surface fats is greater, the more chance there is during collection for an admixture of fat from horny lamellae. Linser (70) stated that relatively pure sebaceous-gland material, with little admixture of horny material, such as the contents of sebaceous cysts and of dermoid cysts, contains insignificant amounts of cholesterol, whereas atheromata, with much horny material in them, have a high cholesterol content. Unna and Golodetz (126, 127) went so far as to distinguish "secretion fats" with a low, and "cell fats" with a high, cholesterol percentage. They found, for instance, 2.8 per cent cholesterol in "comedone fat" (whiteheads) and 19.62 per cent cholesterol in the fat of the horny layer of the soles. When Röhmann (99) was unable to find cholesterol in the sebum of preen glands, which was regarded as the purest "sebaceous material," it was thought that possibly sebaceous glands do not excrete cholesterol at all (104).

This extreme view could not be substantiated. The presence of cholesterol in preen glands was reported qualitatively by Hou (49) and quantitatively by Weitzel (137) as constituting 1.4 per cent of preen-gland fat. Histochemical reactions for cholesterol were

claimed to be positive in mammalian sebaceous glands (p. 331). Nevertheless, it appears to be correct that the major part of cholesterol and its esters in skin surface fats derives from the disintegrating cornifying cells and that the cholesterol content of "pure sebum" is low.

In normal horny cells of the soles and in scales collected from patients with exfoliative diseases, 18–23 per cent of the total lipids are cholesterol (29, 32), while in skin surface fats, which contain both sebaceous-gland and horny-cell lipids, the concentration of cholesterol is only 3–4 per cent. There is more cholesterol on visibly scaling skin surfaces than on normal skin (75). Butcher and Parnell (10), gathering sebum from the forehead after letting it accumulate for 1 week so that plenty of horny fat could have been amassed (p. 310), obtained the highest cholesterol values from an individual with "much dandruff." Kirk (53), who found sebaceous-gland secretion greatly decreased in old women (p. 297), obtained very high cholesterol values in these old women's specimens because, as he interprets it, sebaceous-gland function has decreased but horny-scale formation has not. Furthermore, it seems that the longer one leaves surface fats on the skin, the higher will be their cholesterol content, possibly because gland excretion ceases when saturation has been reached (pp. 288 ff.), while keratinization continues independently of the saturation mechanism. This is well substantiated in the observation of Marchionini and Ottenstein (76): the maximum cholesterol level on the skin surface is reached only 4–5 days after a bath. In contrast, sebaceous-gland excretion comes to a standstill in 3 hours or so. Similar data were recently reported by Carrié also (12).⁷

Thus there can be little doubt that the cholesterol content of skin surface fats depends to a great extent on the proportion in which sebaceous glands and horny layer participate in producing the lipid film. When

7. An extensive critical review of all data on the cholesterol content of human skin surface fats was published by Lincke *et al.* (69; see also 66, 67).

sebum is excreted at a rapid rate, the cholesterol content of the surface film is slow.⁸ When keratinization is accelerated, the cholesterol content of the film is high. With regard to the haphazard nature of the proportion of sebum and keratin production, the long list of quantitative data on the cholesterol content of the film will not be enumerated here. One can hope for consistent results only if one analyzes relatively freshly excreted sebum soon after defatting of the surface. It is probable that such fat contains little, if any, horny-layer fat, because sebaceous excretion goes on at a faster rate than keratinization does.

There is a significant age difference in the cholesterol content of surface fats in man. Eckstein (28) found 9–12 per cent in children's and 1–5 per cent in adults' hair fat. Similar differences were found by Washburn and Liese (133) and by Nicolaides and Rothman (88). The differences are too great and too consistent to be attributed to chance; and that they are due to varying hormonal constellations in the child and adult was proved when it was shown that a hypogonadal male had cholesterol values in the children's range (11.5 per cent) and that this high value dropped to 7.6 per cent after testosterone treatment (133). The relation of cholesterol to squalene content according to age is discussed on page 328. There is no detectable sex difference in the cholesterol content of surface fats (53, 98, 73, 133, 88).

Feeding of cholesterol in large doses increases its relative and absolute amounts in skin surface fats (104). It seems that if there is an excess supply, the regular metabolism of breakdown and resynthesis is by-passed, and surplus cholesterol is taken up from the blood by sebaceous glands and excreted without being metabolized (104).⁹

8. See also the recent publication of H. Lincke, *Beitrag zur Chemie des Hautfettes. III. Der Talg-Cholesterinspiegel*, Arch. f. Dermat. u. Syph., **195**: 540–48, 1953.

9. The cholesterol content of surface lipids is not modified by acne vulgaris in involved areas (R. L. Kile, F. H. Snyder, and J. W. Haeefe, *Nature of skin lipids in acne*, Arch. Dermat. & Syph., **61**: 792–98, 1950).

Because cholesterol on the surface derives to a great extent from cornifying cells, variations in the relation of the free to the esterified fraction reflect variations in the keratinization process rather than changes in sebaceous-gland function. The esterification of cholesterol in the epidermis in the course of keratinization will be discussed on page 486.

According to most data, a considerable portion of cholesterol is esterified in surface fats. In the hair fat of rats the esterified fraction amounts to 20 per cent of the total (27); in human hair fat, to 60 per cent (89); and in wool fat, to about 60 per cent.¹⁰ On the nonhairy skin surface the percentage of esterified cholesterol was over 60 per cent when measured by "dialyzing" the skin with fat solvents (75, 51), and it was 35 per cent in ether wipings of the normal skin of the trunk (103).

The ester/total cholesterol relationship does not change in normal persons if 5–10 gm. of cholesterol are fed by mouth (51), an indication that the esterification of cholesterol is an intrinsic function of the skin (p. 486). However, this cutaneous esterification seems to be under endocrine influence. Lincke (68) found that, after feeding cholesterol to rabbits in which thyroid function was suppressed by large doses of thiouracil, the esterification quotient was much lower than in normal animals. In man, I found that in psoriatic individuals the ratio free/esterified cholesterol in the surface film of uninvolved skin is pathologically low (103).

C. GLYCEROL

The preen-gland fat of ducks contains only 1.5 per cent glycerol (137), and wool fat contains none (26). Its occurrence in human skin surface fat as glyceride was demonstrated by Zehender (146) in material from the forehead and by MacKenna *et al.* (73) in material from the forearms. The latter calculated that about 25 per cent of the total surface fat is triglycerides. The calculations of Kvorning (58) suggested

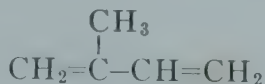
10. See E. S. Lower, *Lanolin*, Manufact. Chemist, **15**: 13–16, 35, 68–71, 1945.

even more glycerides: when assuming that glycerol is present as tristearate, he found that 50 per cent of the total lipid weight was accounted for by triglycerides. Kvorning, however, manipulated extremely small amounts of sebum, and his data should be rechecked on a macroscale.

In my laboratory Nicolaides (unpublished) found 0.71 per cent glycerol in the fat from one sample of stored hair, which corresponds to about 7 per cent triglycerides. However, three other samples gave an average value of only 0.17 per cent, corresponding to about 1.7 per cent triglycerides. Subcutaneous fat, which is nearly all triglyceride, contains about 10 per cent glycerol. The low value of glycerides found is probably due to lipolytic hydrolysis during storage. If the hair is extracted after hydrolysis has occurred, the free glycerol will not be taken up by the fat solvents during extraction of the hair, since glycerol is insoluble in these solvents.

D. TRITERPENOID ALCOHOLS

In a more restricted sense the term "terpene" is used to designate compounds with 10 carbon atoms derived from the basic formula $C_{10}H_{16}$. These hydrocarbons occur mainly in essential oils of plants. Terpenes have a distinct architectural and chemical relation to the simpler isoprene (or isopentene) molecule, C_5H_8 :



Three terpene molecules or 6 isoprene units connected form triterpenes with the general formula $C_{30}H_{48}$. Compounds having a more distant connection with terpenes but still containing features which link them to terpene structures are called "terpenoids" or "isoprenoids," in analogy with the term "steroid," which includes, in addition to sterols, more remotely connected relatives (40).

In a remarkably painstaking study from 1870 to 1873 E. Schulze (116) isolated a hitherto unknown substance from wool fat.

In addition to characteristic cholesterol crystals in the unsaponifiable portion, he noted gelatinous flocks, which he separated from cholesterol by preparing the respective benzoic acid esters. He regarded the new material as closely related to cholesterol and called it "ischolesterol." The findings of Schulze have often been confirmed, and different methods have been recommended for the preparation and purification of "ischolesterol" (65, 26, 37). Relatively early, some doubt was expressed as to whether "ischolesterol" was a well-defined single chemical compound (100); but the first thorough and successful analysis was made as late as 1930 by Windaus and Tschesche (143), who conclusively demonstrated that "ischolesterol" is not a chemical individual but a mixture.

They isolated two substances from "pure" ischolesterol: (a) lanosterol, with the empirical formula $C_{30}H_{50}O$, and (b) agnosterol, with the empirical formula $C_{30}H_{48}O$, lanosterol constituting about 92 per cent and agnosterol about 8 per cent of "ischolesterol." Further work (144, 22, 23, 77) confirmed and enlarged these findings. The empirical formulae containing 30 carbon atoms indicated that neither compound is isomeric with cholesterol, which contains 27. The data rather pointed toward a relationship with some resin alcohols of plant origin belonging to the group of triterpenes. The crucial experiments were made by H. Schulze (117) and by Dorée and Petrow (25), who obtained from lanosterol the characteristic reduction product of triterpenes.

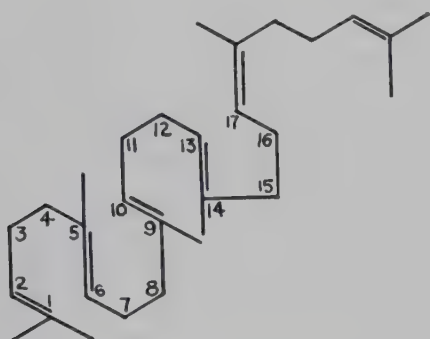
Ruzicka and his co-workers (112, 111, 110), further analyzing ischolesterol, found that, in addition to lanosterol and agnosterol, it also contains a dihydro derivative and an optical isomer of lanosterol: dihydro-lanosterol and γ -lanosterol. The latter is identical with dihydroagnosterol, which was prepared earlier by Marker *et al.* (77). A surprising finding has been that dihydro-lanosterol proved to be identical with the dihydro derivative of kryptosterol (110), a triterpenoid compound isolated by H. Wieland (139) from yeast.

After extensive chemical work over a period of many years (5, 138, 132) it is now definitely established (131, 15) that lanosterol has the same ring structure as cholesterol. Its secondary alcohol group can be oxidized to a ketone group, so that lanostenone results, in analogy to the formation of cholestenone from cholesterol. On chemical grounds, Ruzicka (131) has lately classified lanosterol as belonging to the steroids rather than to the triterpenoids.

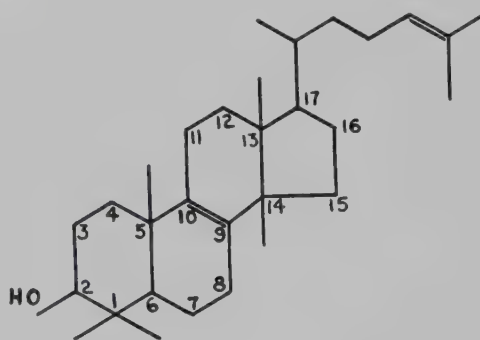
Since squalene has been isolated from human skin surface fats (p. 328), the simi-

cholesterol is achieved by loss of an angular methyl group at position 14 and two more at 1 (the numbering here employed is not that customarily given for cholesterol—see p. 323) (145).

The occurrence of lanosterol and related compounds in skin surface fats is restricted to a few species of ruminants, viz., sheep, goat, llama, and dromedary (63). None was found in cattle, rabbits, and man (63, 24, 74). It is a specific metabolic product of the skin, not found in the internal organs of sheep (62).



Structural formula of squalene
(145), $C_{30}H_{50}$



Structural formula of lanosterol
(131), $C_{30}H_{50}O$

FIG. 7.—Squalene and lanosterol

ilarity between the structures of lanosterol and squalene deserves particular attention, because these are two substances which were isolated in considerable amounts from wool fat and from human skin surface fat, respectively (Fig. 7). Comparison of the two structures indicates that lanosterol can be regarded as a cyclization product of squalene, with the substitution of an alcohol group in position 2. Only the transfer of one methyl group from position 9 in squalene into position 13 in lanosterol has to be postulated, which could well be effected by way of a methylene bridge (145).

A further biologically important point is the close relationship of lanosterol to cholesterol. Conversion to the carbon skeleton of

Much emphasis has been placed on the species-specificity of the alcohols here described (63, 62, 61, 134, 74). It is indeed remarkable that such large amounts occur in the hair fat of some species and are entirely absent in others. Still it should be pointed out that the components of "ischolesterol" are closely related to, or identical with, cyclization products of squalene and that the specificity of the biochemical synthesis in the sheep is restricted to the ability of its skin to form alcohols of the basic common skeleton, while the same skeleton remains a group of hydrocarbons in the skin and hair fats of humans. Traces of "ischolesterol" were found by Sobel (119) in human dermoid fat.

VI. HYDROCARBONS

A. SQUALENE

Squalene is an aliphatic acyclic hydrocarbon with the empirical formula $C_{30}H_{50}$. It consists of six isoprene units (Fig. 7). This compound was first isolated from the unsaponifiable fraction of the liver oil of sharks (Squalidae) in 1916 (125), but it was not until 1926 that its chemical constitution was established (42).¹¹ Since then it has been shown that squalene is present not only in

TABLE 5
SQUALENE IN SEBUM AND SEBUM-
LIKE MATERIALS

Author	Material	Per Cent Squalene in Total Lipid (Av. Values)
Sobel (119)	Human dermoid fat	11.4
	Human hair fat	3.9
	Human cerumen	5.5
	Human smegma	1.0
MacKenna <i>et al.</i> (73)	Human sebum from forearm	5.0
Nicolaidis <i>et al.</i> (88)	Human hair fat, adult males	5.5
	Human hair fat, adult females	6.0
	Human hair fat, boys 6-12 years	1.4

fish-liver oil but also in many vegetable oils (122).

Dimter (19) was the first to show that squalene is produced by the skin in man, when he isolated it from the unsaponifiable fraction of human ovarian dermoid cysts. He showed that squalene is a primary product of these cysts and is not formed secondarily by chemical manipulations. Dimter was unable to find squalene in the normal human organism, although he searched for it in

11. This formula was confirmed by synthesis in 1931 by Karrer *et al.* (P. Karrer and A. Helfenstein, *Synthese des Squalens*, *Helvet. chim. acta*, **14**:78-85, 1931).

depot fat, in blood serum, and in the liver fat of feti and adults.

Recently, the presence of squalene was demonstrated in normal skin surface fats of humans. Sobel (119) found it in human hair fat, human cerumen, human smegma, and in horse smegma (Table 5). MacKenna, Wheatley, and Wormall (73) isolated it from skin fats of the human forearm. In addition to unchanged squalene, analyses indicated the presence of cyclized derivatives of squalene (119, 74); but so far their structures have not been elucidated (73).

Since its discovery in fish-liver oil, there has been extensive discussion as to whether squalene may be regarded as a precursor of cholesterol during its biosynthesis. Only recently was conclusive evidence presented by Langdon and Bloch (60) that squalene is converted into cholesterol by the animal organism. Nicolaidis and Rothman (88) found that, while in children the cholesterol content of hair fat is about three times greater than in adults (p. 325), squalene, just the opposite, is three to four times higher in the adult than in children. Thus, if squalene is an obligatory intermediate in the biosynthesis of cholesterol, children carry out the synthesis much more efficiently than adults. The cholesterol to squalene molar ratio was found to be twelve to eighteen times as high in children as it is in adults (88).¹²

A higher homologue of squalene, called "hepene," was found in human livers by Dimter (20) and was considered as the possible aliphatic precursor of cholesterol (120). Dimter (20) found an increase in hepene and a decrease in cholesterol in the liver of adult organisms, as compared with the young, changes resembling those observed in human hair fat; and similar findings were recorded concerning the squalene content of the shark-liver oil in the young and adult.

12. For synthesis of cholesterol by skin tissue see chap. 20, p. 487.

Thus, apparently, the higher efficiency of the juvenile organism in converting squalene into cholesterol appears to be a general phenomenon (88).

While no "ischolesterol" is found in human skin fats, no squalene or only traces of squalene (119) are found in wool fat. These species differences remind one of the species differences in fatty acids. Indeed, it seems that the chemical composition of sebaceous gland and other skin products shows more striking species differences among mammals than that of any other organ or organ product.

B. OTHER HYDROCARBONS

Wool fat contains about 1 per cent hydrocarbons (124); and Lederer (130) showed that this is not an accidental contamination of the fat but a primary product of sheep's skin. The hydrocarbon content of human skin surface fats is much higher than that, and again there is little doubt that these hydrocarbons are natural products manufactured by the human skin.¹³ In the analyses of Wheatley (134) the total amount of hydrocarbons in the surface fat of the forearm, excluding squalene and cyclization products of squalene, is 7.5 per cent. MacKenna, Wheatley, and Wormall (74) isolated a saturated hydrocarbon with the molecular weight of 428, which approximately corresponds with a chain length of C_{30} .

Hydrocarbons, other than squalene, could be demonstrated also in hair fat collected from the scalp. According to the analyses of Nicolaides (89), straight-chain and branched-chain saturated hydrocarbons are present. The straight-chain portion, isolated by the urea-separation method, was found to be a homologous series by mass spectrography. The chain lengths of the members of this series have not yet been definitely established. One sample gave a series ranging from C_{15} to C_{40} , with odd as well as even members present. These results, however, require further confirmation. No unsaturated straight-chain compounds have been found. The branched-chain saturated hydrocarbons have not yet been worked up. The branched-chain unsaturated compounds were found to have lower iodine values than squalene, thus indicating the presence of compounds other than squalene. A saturated hydrocarbon with a molecular weight corresponding to C_{40} was reported to occur in forearm sebum by Festenstein and Morton (33).

A most interesting hydrocarbon giving an immediate Liebermann-Burchard color reaction, first isolated by Dimter (21) in human serum, was detected in human skin surface fat by MacKenna *et al.* (74) and was isolated by Nicolaides (89). This compound might be an intermediate in the conversion of squalene to cholesterol (21).

VII. VITAMINS AND VITAMIN PRECURSORS

A. PRO-VITAMIN D

The occurrence of vitamin D precursors in skin surface fats was postulated since it has been known that ultraviolet irradiations cure rickets. A great number of biological experiments supported the view that vitamin D is formed on the skin surface or in the most superficial skin layers from its precursors by ultraviolet light and is absorbed through the skin. The experiments of Hou on ducks were mentioned on page 285. Pro-vitamin D_3 (7-dihydrocholesterol) was iso-

lated from pig skin by Windaus and Bock (140) and from chicken skin by Koch and Koch (55). Helmer and Jansen (43) showed that irradiated human sebum has antirachitic properties. However, nobody has succeeded as yet in demonstrating, directly by chemical means, the presence of vitamin D precursors either in wool fat or in human skin surface fats. In wool fat its presence was claimed by Hess, Weinstock, and Helman (46), but this was found to be erroneous by Drummond and Baker (26).

In human skin a claim has been made for the presence of ergosterol, which is a pre-

13. According to recent analyses of Nicolaides, external contamination is still a possibility (see n. 6).

cursor of vitamin D in plants but does not occur in animals (44). The recent thorough investigations of MacKenna's group (74, 33) leaves little doubt that the known precursors of vitamin D cannot be demonstrated in human skin surface fats by chemical or physical means. The discrepancy in the results of biological experimentation and chemical analyses is puzzling. MacKenna *et al.* (74) consider the possibility of the existence of a hitherto unknown pro-vitamin D. They have some support of their view in having isolated a substance somewhat similar to 7- α -hydroxycholesterol, the reactions of which are similar in some respects to the known D pro-vitamins but differ in other respects.

B. VITAMIN A AND PRECURSORS

A similar problem presents itself in regard to vitamin A and its precursor, β -carotene. Cornbleet and Popper (14) were unable to demonstrate the presence of vitamin A in human skin, and their negative results were confirmed by MacKenna *et al.* (74) and by Festenstein and Morton (33) also concerning β -carotene. Vitamin A is required for the maintenance of normal epidermal cell life (p. 382), and it is not feasible that it should

not reach the epidermis. The probability is that it is present in a masked (conjugated?) form, in which it escapes chemical demonstration. That vitamin A does occur in a chemically undetectable form has been known.

C. VITAMIN E

In their first publication MacKenna *et al.* (73) reported on the presence of significant amounts of vitamin E in human skin surface fats. But later they found (74, 33) that most of the ferric chloride-reducing substances in sebum are different from vitamin E and that probably not more than 0.2 mg/gm sebum is α -tocopherol. These authors presented supporting evidence for the view that the substances reducing ferric chloride may serve as antioxidants in sebum. Feeding tocopherols to persons, they did not obtain a significant increase of ferric chloride-reducing substances in the surface fats.

D. OTHER VITAMINS

No vitamin K and none of the water-soluble vitamins could be demonstrated on the human skin surface of the forearm when this was bathed in acetone (74, 33). For vitamin B factors in the aqueous surface film see chapter 7, page 211.

VIII. HISTOCHEMICAL OBSERVATIONS

The progressive fatty metamorphosis in sebaceous glands can be followed with a great number of different fat stains. The accumulation of fat globules follows a certain pattern, and Suskind (121) found that this pattern is grossly exaggerated when sebum excretion is pathologically augmented, as it is in acne vulgaris and chlorine acne.

Attempts to identify single lipid components in sebaceous-gland cells and in sebum by histochemical reactions have often been made, but in spite of painstaking work and accurate observations no conclusive results have been obtained. All authors have emphasized the limitations in the histochemical differentiation of lipids (104, 121, 83).

Montagna *et al.*, studying sebaceous glands of rats (83), hamsters (82), and human glands of the external ear canal (84), described the presence of phospholipids in the peripheral matrix cells of sebaceous-gland acini and their gradual disappearance with a progressing fatty infiltration. Suskind (121) confirmed these findings for human sebaceous glands and observed that the phospholipids are linked with cytoplasmic granules and rodlets, presumably Golgi bodies or mitochondria. Previously, Melczer and Deme (78) had postulated that the Golgi bodies play a role in the glandular activity of sebaceous glands and described in great detail the morphological changes which these bodies undergo as the cells be-

come infiltrated with fat globules. Essentially, there is a disintegration into a granular mass.

If these histochemical findings are accepted as indicating the destruction of phospholipids during sebum formation, one can state that this process is analogous to what happens during keratinization. The active matrix cells are rich in phospholipids, but in the course of their natural life-cycle, which ends with their death, these phospholipids disappear (p. 488). Phospholipids are not present in skin surface fats.

Melczer thought that sebaceous glands are stimulated by the injection of pilocarpine and noticed that in such "stimulated" glands the formation of the first phases of the Golgi bodies, the so-called "presubstance," is greatly augmented. Similarly, Suskind (121) found that the granules and rodlets are increased in number in hyperactive glands of acne vulgaris and chlorine acne.

Except for phospholipids, no other chemical constituents could be identified conclusively. Glycerides with unsaturated fatty acids and cholesterol esters were tentatively identified (83, 121). The presence of free cholesterol in normal glands could be excluded with fair certainty, because no digitonin-precipitable anisotropic material was found (83). However, free cholesterol seems to be present in comedones and in acne cysts (121); in such lesions cholesterol and its esters are probably epidermal rather than glandular products (p. 324).

On the basis of histochemical findings, Melczer and Deme (79) postulated the presence of three different layers of lipids in the acini: an outer layer composed of fatty acids, a middle zone of long-chain aliphatic alcohols, and an inner layer of neutral fats; they thought that these three layers fused when the material arrived in the duct. It was thought, however (83, 121), that such a detailed interpretation of otherwise most remarkable histochemical findings depends on

too many uncertain and questionable assumptions.

That sebum contains fluorescent material was demonstrated histochemically (83).

Rather contradictory results were obtained concerning the histochemical demonstration of enzymes. Sebaceous glands have abundant alkaline phosphatase in the rat (83), but there is only a little in the hamster (82) and none in man (121). Acid phosphatase was found in the glands of the hamster (82). The presence of phosphatases in the basal cells would account for the decomposition of phospholipids with liberation of fatty acids.

Signs of lipase activity were seen in sebaceous glands of mice (56) and of hamsters in the cells undergoing breakdown and in the sebum (82). But none or only traces were found in the rat (83) and in man (39).

The histochemistry of the preen gland was studied extensively by Cater and Lawrie.¹⁴ They found two distinctly different zones in the gland, a central one with high acid phosphatase and glycogen content ("glycogen zone") and a peripheral one with no glycogen granules and no acid phosphatase ("sebaceous zone"). In the glycogen zone lipids were found in the luminal cells, while in the sebaceous zone the lipid droplets were present in the peripheral flattened cells lying against the connective-tissue stroma. The authors discussed the possible separate function of these structurally different zones and pointed out some interesting analogies to the histochemistry of the developing feathers, and mammalian hairs and sebaceous glands. An esterase, which splits short-chain fatty-acid esters, was found in the preen gland, but no true lipase and no cholinesterase could be demonstrated.

14. D. B. Cater and N. R. Lawrie, Some histochemical and biochemical observations on the preen gland, *J. Physiol.*, **111**:231-43, 1950; A histochemical study of the developing preen glands of chick, fourteenth day of incubation until fourteen days after hatching, *ibid.*, **112**:405-19, 1951.

BIBLIOGRAPHY

1. ABRAHAM, E. E. V., and HILDITCH, T. P. The acidic components of wool grease, *J. Soc. Chem. Indust.*, **54**:398T-404T, 1935.
2. AMESSEDER, F. Über den "Cetylalkohol" aus Dermoidcysten Fett, *Ztschr. f. physiol. Chem.*, **52**:121-28, 1907.
3. ANDERSON, R. J. Chemistry of the lipids of tubercle bacilli, *Harvey Lect.*, **35**:271-313, 1940.
4. BEHRING, H. VON. Enthalten Bakterien Sterine? *Ztschr. f. physiol. Chem.*, **192**:112-13, 1930.
5. BELLAMY, L. J., and DORÉE, C. Lanosterol; the oxidation of lanosterol with chromic acid, *J. Chem. Soc. (London)*, 1941, pp. 172-81.
6. BURR, G. O., and BURR, M. M. On the nature and role of the fatty acids essential in nutrition, *J. Biol. Chem.*, **86**:587-621, 1930.
7. BURTENSHAW, J. M. L. Mechanism of self-disinfection of human skin and its appendages, *J. Hyg.*, **42**:184-210, 1942.
8. ———. Self-disinfection of the skin, *Brit. M. Bull.*, **3**:161-64, 1945. See also The autogenous disinfection of the skin. *In*: MACKENNA, Modern trends in dermatology, pp. 158-85. London: Butterworth & Co., Ltd., 1948.
9. BUTCHER, E. O., and COONIN, A. The physical properties of human sebum, *J. Invest. Dermat.*, **12**:249-54, 1949.
10. BUTCHER, E. O., and PARNELL, J. P. Sebaceous secretion on the human head, *J. Invest. Dermat.*, **9**:67-74, 1947.
11. ———. The distribution and factors influencing the amount of sebum on the skin of the forehead, *ibid.*, **10**:31-38, 1948.
12. CARRIÉ, C. Untersuchungen über die Lipide der Hautoberfläche, *Arch. f. Dermat. u. Syph.*, **188**:241-58, 1949-50.
13. CATCH, J. R.; JONES, T. S. G.; and WILKINSON, S. The chemistry of polymyxin A ("Aerosporin"); isolation of the amino acids D-leucine, L-threonine, L- α - γ -diaminobutyric acid, and an unknown fatty acid, *Biochem. J.*, **43**:xxvii-xxviii, 1948.
14. CORNBLEET, T., and POPPER, H. Properties of human skin revealed by fluorescence microscopy: the normal skin; the vitamin A content of the skin, *Arch. Dermat. & Syph.*, **46**:59-65, 1942.
15. CURTIS, R. G.; FRIDRICHSON, J.; and MATHIESON, A. McL. Structure of lanosterol, *Nature*, **170**:321-22, 1952.
16. DANIEL, D.; LEDERER, E.; and VELLUZ, L. Sur les constituants de la graisse de laine. II. Étude chromatographique de la partie insaponifiable, *Bull. Soc. chim. biol.*, **27**:218-25, 1945.
17. DARMSTÄDTER, L., and LIFSCHÜTZ, J. Beiträge zur Kenntnis der Zusammensetzung des Wollfettes, *Ber. d. deutsch. chem. Gesellsch.*, **29**:618-22, 1474-77, and 2890-2900, 1896.
18. DE SANCTIS, 1894. Quoted by VELLUZ and LEDERER (130).
19. DIMTER, A. Untersuchungen über das Unverseifbare. I. Über das Unverseifbare des Ovarialdermoidcystenfettes, *Ztschr. f. physiol. Chem.*, **270**:247-65, 1941.
20. ———. Untersuchungen über das Unverseifbare. II. Das Unverseifbare aus den fetalen Lebern und den Lebern Erwachsener, *ibid.*, **271**:293-315, 1941.
21. ———. Untersuchungen über das Unverseifbare; über das Unverseifbare des menschlichen Serums, *ibid.*, **272**:189-200, 1942.
22. DORÉE, C., and GARRATT, D. C. Lanosterol and a new method for its preparation, *J. Soc. Chem. Indust.*, **52**:141T-143T, 1933.
23. ———. Isocholesterol: the chemistry of lanosterol, *ibid.*, pp. 355T-361T.
24. DORÉE, C.; MCGHIE, J. F.; and KURZER, F. Lanosterol; hydrocarbons formed by the action of dehydrating agents, *J. Chem. Soc. (London)*, 1947, pp. 1467-71.
25. DORÉE, C., and PETROW, V. A. Lanosterol, *J. Chem. Soc. (London)*, 1936, pp. 1562-67.
26. DRUMMOND, J. C., and BAKER, L. C. Composition of wool fat, *J. Soc. Chem. Indust.*, **48**:232T-238T, 1929.
27. ECKSTEIN, H. C. The cholesterol content of the hair of the albino rat, *Proc. Soc. Exper. Biol. & Med.*, **23**:581, 1926.
28. ———. The cholesterol content of hair, wool, and feathers, *J. Biol. Chem.*, **73**:363-69, 1927.
29. ECKSTEIN, H. C., and WILE, U. J. The cholesterol and phospholipid content of the cutaneous epithelium of man, *J. Biol. Chem.*, **69**:181-86, 1926.
30. EMANUEL, S. Quantitative determinations of the sebaceous gland's function,

- with particular mention of the method employed, *Acta dermat.-venereol.*, **17**:444-56, 1936.
31. ———. Mechanism of sebum secretion, *ibid.*, **19**:1-18, 1938.
 32. ENGMAN, M. F., and KOOYMAN, D. J. Lipids of the skin surface. LXVII, *Arch. Dermat. & Syph.*, **29**:12-19, 1934.
 33. FESTENSTEIN, G. N., and MORTON, R. A. Spectrophotometric studies on human sebum, *Biochem. J.*, **52**:168-77, 1952.
 34. FIESER, L. F. A companion of cholesterol, *J. Am. Chem. Soc.*, **73**:5007, 1951.
 35. FIESER, L. F., and FIESER, M. Natural products related to phenanthrene. ("American Chemical Society Monographs," No. 70.) 3d ed. New York: Reinhold Publishing Corp., 1949.
 36. FRENEY, M. R. Studies on the merino fleece; the chemistry of suint, *J. Soc. Chem. Indust.*, **53**:131T-134T, 1934.
 37. ———. Studies on the merino fleece; a rapid method of separating cholesterol and isocholesterol of wool wax, *ibid.*, pp. 289T-290T.
 38. GILLESPIE, D. T. C. Wool wax, *J. Textile Inst.*, **39**:P45-P85, 1948.
 39. GOMORI, G. Personal communication.
 40. GUENTHER, E. The essential oils, Vol. 1. Toronto, London, and New York: D. Van Nostrand Co., 1948.
 41. HAUSER, F. Personal communication.
 42. HEILBRON, I. M.; HILDITCH, T. P.; and KAMM, E. D. The unsaponifiable matter from the oils of elasmobranch fish; the hydrogenation of squalene in the presence of nickel, *J. Chem. Soc. (London)*, 1926, pp. 3131-36.
 43. HELMER, A. C., and JANSEN, C. H. Vitamin D precursors removed from human skin by washing, *Studies Inst. Divi Thomae*, **1**:207-16, 1937.
 44. HENTSCHEL, H., and SCHINDEL, L. Ergosterinnachweis in menschlicher Haut, *Klin. Wchnschr.*, **9**:262, 1930.
 45. HERRMANN, F., and PROSE, P. H. Studies on the ether-soluble substances on the human skin. I. Quantity and "replacement sum," *J. Invest. Dermat.*, **16**:217-30, 1951.
 46. HESS, A. F.; WEINSTOCK, M.; and HELMAN, F. D. Antirachitic value of irradiated cholesterol and phytosterol; factors influencing its biological activity, *J. Biol. Chem.*, **63**:305-8, 1925.
 47. HORN, D. H. S., and HOUGEN, F. W. The isolation of some homologous higher molecular weight glycols from the unsaponifiable fraction of wool wax, *Chemistry & Industry*, 1951, p. 670.
 48. HORN, D. H. S.; HOUGEN, F. W.; and RUDLOFF, E. VON. Constitution of the hydroxy acids of wool wax, *Chemistry & Industry*, 1952, p. 106.
 49. HOU, H. C. Studies on the glandula uropygialis of birds, *Chinese J. Physiol.*, **2**:345-80, 1928.
 50. IDLER, D. R., and BAUMANN, C. A. Skin sterols. II. Isolation of Δ^7 -cholestenol, *J. Biol. Chem.*, **195**:623-28, 1952.
 51. INCEDAYI, C. K., and OTTENSTEIN, B. Neuere Untersuchungen über die Beziehungen zwischen Psoriasis und Lipoidosen, *Acta dermat.-venereol.*, **21**:674-98, 1941.
 52. JONES, K. K.; SPENCER, M. C.; and SANCHEZ, S. A. The estimation of rate of secretion of sebum in man, *J. Invest. Dermat.*, **17**:213-26, 1951.
 53. KIRK, E. Quantitative determinations of the skin lipid secretion in middle-aged and old individuals, *J. Gerontol.*, **3**:251-66, 1948.
 54. KLIGMAN, A. M., and GINSBERG, D. Immunity of adult scalp to infection with *Microsporum audouini*, *J. Invest. Dermat.*, **14**:345-58, 1950.
 55. KOCH, E. M., and KOCH, F. C. The provitamin D of the covering tissue of chickens, *Poultry Sc.*, **20**:33-35, 1941.
 56. KUNG, S. K. Lipase activity during experimental epidermal carcinogenesis, *J. Nat. Cancer Inst.*, **9**:435-38, 1949.
 57. KUWATA, T., and ISHII, Y. Investigations on wool fat, *J. Soc. Chem. Indust. Japan*, **39**:317-19 and 358-59, 1936.
 58. KVORNING, S. A. Investigations into the pharmacology of the skin fats and of ointments. IV. Investigations into the composition of the lipids on the skin of normal individuals, *Acta pharmacol. et toxicol.*, **5**:383-96, 1949.
 59. ———. Analyses of the cutaneous lipids, *Excerpta med.*, Sec. XIII, **6**:288, 1952.
 60. LANGDON, R. G., and BLOCH, K. The biosynthesis of squalene and cholesterol, *J. Am. Chem. Soc.*, **74**:1869-70, 1952; see also The biosynthesis of squalene, *J. Biol. Chem.*, **200**:129-34, 1953; and The utilization of squalene in the biosynthesis of cholesterol, *ibid.*, pp. 135-44.
 61. LEDERER, E. Graisse de cheveux et graisse

- de laine, Industrie de la parfumerie, 5: 552-54, 1950.
62. LEDERER, E., and MERCIER, O. Sur les constituants de la graisse de laine; la peau de mouton comme organe de la biosynthèse des alcools triterpéniques, *Biochim. et biophys. acta*, 2:91-94, 1948.
 63. LEDERER, E., and TCHEN, P. K. Sur les constituants de la graisse de laine. III. Dosage de l' "isocholestérol" (lanostérol, agnostérol etc.), *Bull. Soc. chim. biol.*, 27: 419-24, 1945.
 64. LIFSCHÜTZ, J. Zur Kenntnis des Meta-cholesterins und seiner Nebenprodukte, *Biochem. Ztschr.*, 129:115-27, 1922.
 65. LIFSCHÜTZ, J., and VIETMEYER, O. Iso-phytosterin im Kaugummi, *Ztschr. f. physiol. Chem.*, 155:240-44, 1926.
 66. LINCKE, H. Beiträge zur Chemie und Biologie des Hautoberflächenfettes, *Arch. f. Dermat. u. Syph.*, 188:453-81, 1949.
 67. ———. Einige Bemerkungen zu den "Untersuchungen über die Lipide der Hautoberfläche" von C. Carrié, *ibid.*, 190:203-8, 1950.
 68. ———. Über den Einfluss verfütterten Cholesterins auf den Cholesteringehalt des Hautoberflächenfettes beim Kaninchen, *Dermatologica*, 104:71-79, 1952.
 69. LINCKE, H., and KLÄWL, K. Zur Cholesterinbestimmung im Hautfett, *Arch. f. Dermat. u. Syph.*, 192:402-22, 1951.
 70. LINSER, P. Über den Hauttalg beim gesunden und bei einigen Hauterkrankungen, *Deutsches Arch. f. klin. Med.*, 80:201-24, 1904.
 71. LIPSON, M. Chemical investigations on wool wax, *J. Council Scient. & Indust. Research (Australia)*, 13:273-77, 1940.
 72. LUSTIG, B., and PERUTZ, A. Über die Beeinflussung des Oberhautfettes durch Fettzufuhr, *Biochem. Ztschr.*, 249:370-72, 1932.
 73. MACKENNA, R. M. B.; WHEATLEY, V. R.; and WORMALL, A. The composition of the surface skin fat ("sebum") from the human forearm, *J. Invest. Dermat.*, 15:33-47, 1950.
 74. ———. Studies of sebum. 2. Some constituents of the unsaponifiable matter of human sebum, *Biochem. J.*, 52:161-68, 1952.
 75. MARCHIONINI, A.; MANZ, E.; and HUSS, F. Der Cholesteringehalt der Hautober-schicht bei der Seborrhoe und bei der Psoriasis. Beiträge zur Kenntnis der pathochemischen Hautkonstitution des Status seborrhoicus, *Arch. f. Dermat. u. Syph.*, 176:613-45, 1938.
 76. MARCHIONINI, A., and OTTENSTEIN, B. Stoffwechselveränderungen im Schwitzbad bei Hautgesunden und Hautkranken, *Klin. Wchnschr.*, 10:969-74, 1931.
 77. MARKER, R. E.; WITTLE, E. L.; and MIXON, L. W. Sterols. XVI. Lanosterol and agnosterol, *J. Am. Chem. Soc.*, 59: 1368-73, 1937.
 78. MELCZER, N., and DEME, S. Beiträge zur Tätigkeit der menschlichen Talgdrüsen. II. Rolle und Formveränderungen des Golgi-Apparates während der Talgproduktion, *Arch. f. Dermat. u. Syph.*, 183:388-95, 1942.
 79. ———. Beiträge zur Aktivität der menschlichen Talgdrüsen. I. Histochemische Veränderungen während der Produktion von Talg, *Dermatologica*, 86:24-36, 1942.
 80. MOHS, P. Welche Sterine sind im Wollfett enthalten? *Fette u. Seifen*, 45:152-54, 1938.
 81. MONTAGNA, W. Histochemistry of the sebaceous gland of the rat, *Anat. Rec.*, 97: 356, 1947.
 82. MONTAGNA, W., and HAMILTON, J. B. Histochemical analysis of the sebaceous glands of the hamster, *Federation Proc.*, 6:166-67, 1947.
 83. MONTAGNA, W., and NOBACK, C. R. Histochemical observations on the sebaceous glands of the rat, *Am. J. Anat.*, 81:39-60, 1947.
 84. MONTAGNA, W.; NOBACK, C. R.; and ZAK, F. G. Pigment, lipids and other substances in the glands of the external auditory meatus of man, *Am. J. Anat.*, 83:409-36, 1948.
 85. MOORE, P. R., and BAUMANN, C. A. Skin sterols. I. Colorimetric determination of cholesterol and other sterols in skin, *J. Biol. Chem.*, 195:615-21, 1952.
 86. MURPHY, J. C., and ROTHMAN, S. Artificially induced resistance of *Trichophyton gypseum* to pelargonic acid, *J. Invest. Dermat.*, 12:5-6, 1949.
 87. MURRAY, K. E., and SCHOENFELD, R. The aliphatic alcohols of wool wax. V. Studies in waxes, *J. Am. Oil Chem. Soc.*, 29:416-20, 1952.
 88. NICOLAIDES, N., and ROTHMAN, S. Studies on the chemical composition of human hair fat. I. The squalene-cholesterol relationship in children and adults, *J. Invest. Dermat.*, 19:389-91, 1952.
 89. ———. Studies on the chemical composi-

- tion of human hair fat. II. The overall composition with regard to age, sex and race, *ibid.*, **21**:9-14, 1953.
90. OHLE, H. Einfache Kohlenstoffverbindungen des Fettreihe. In: OPPENHEIMER, Handb. d. Biochem., **1**:33. 2d ed. Jena: G. Fischer, 1924.
 91. PARNAS, J. Über fermentative Beschleunigung der Cannizaroschen Aldehydumlagerung durch Gewebssäfte, Biochem. Ztschr., **28**:274-94, 1910.
 92. PECK, S. M.; ROSENFELD, H.; LEIFER, W.; and BIERMAN, W. Role of sweat as a fungicide, Arch. Dermat. & Syph., **39**:126-48, 1939.
 93. PERUTZ, A., and LUSTIG, B. Zur Physiologie der Fettausscheidung an der Hautoberfläche, Biochem. Ztschr., **261**:128-31, 1933.
 94. PILLSBURY, D. M., and REBELL, G. The bacterial flora of the skin, J. Invest. Dermat., **18**:173-86, 1952.
 95. REBELL, G.; PILLSBURY, D. M.; SAINT-PHALLE, M. DE; and GINSBURG, D. Factors affecting the rapid disappearance of bacteria placed on the normal skin, J. Invest. Dermat., **14**:247-63, 1950.
 96. REISS, F. Discussion on KLIGMAN and GINSBERG (54).
 97. REISS, F., and LUSTIG, B. Evaluation of fungicides, Dermatologica, **97**:312-19, 1948.
 98. RICKETTS, C. R.; SQUIRE, J. R.; and TOPLEY, E. Human skin lipids with particular reference to the self-sterilizing power of the skin, Clin. Sc., **10**:89-111, 1951.
 99. RÖHMANN, F. Über das Sekret der Bürzeldrüsen, Hofmeisters Beitr. z. chem. Physiol. u. Path., **5**:110-32, 1904.
 100. ———. Beiträge zur Kenntnis der Bestandteile des Wollfettes, Biochem. Ztschr., **77**:299-328, 1916.
 101. ROTHMAN, S. Discussion on KLIGMAN and GINSBERG (54).
 102. ———. Susceptibility factors in fungus infections in man, Tr. New York Acad. Sc., **12**:27-33, 1949.
 103. ———. Abnormalities in the chemical composition of the skin surface film in psoriasis, Arch. Dermat. & Syph., **62**:814-19, 1950.
 104. ROTHMAN, S., and SCHAAF, F. Die Chemie der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1/2**: 161-377. Berlin: J. Springer, 1929.
 105. ROTHMAN, S.; SMILJANIC, A. M.; and SHAPIRO, A. L. Fungistatic action of hair fat on *Microsporon audouini*, Proc. Soc. Exper. Biol. & Med., **60**:394-95, 1945.
 106. ROTHMAN, S.; SMILJANIC, A. M.; SHAPIRO, A. L.; and WEITKAMP, A. W. The spontaneous cure of tinea capitis in puberty, J. Invest. Dermat., **8**:81-98, 1947.
 107. ROTHMAN, S.; SMILJANIC, A. M.; and WEITKAMP, A. W. Mechanism of spontaneous cure in puberty of ringworm of the scalp, Science, **104**:201-3, 1946.
 108. ROTHMAN, S., and SULLIVAN, M. B. Amino acids on the normal skin surface, J. Invest. Dermat., **13**:319-21, 1949.
 109. RUDLOFF, E. VON. Fatty alcohols from wool wax unsaponifiable by urea-complex formation, Chemistry & Industry, 1951, pp. 338-39.
 110. RUZICKA, L.; DENSS, R.; and JEGER, O. Zur Kenntnis der Triterpene; Beweis der Identität von Lanosterin und Kryptosterin, Helvet. chim. acta, **28**:759-66, 1945.
 111. RUZICKA, L.; REY, E.; and MUHR, A. C. Zur Kenntnis der Triterpene; über verschiedene Umwandlungsprodukte des Lanosterins, Helvet. chim. acta, **27**:472-89, 1944.
 112. RUZICKA, L.; REY, E.; SPILLMANN, M.; and BAUMGARTNER, H. Zur Kenntnis der Triterpene; über weitere Umwandlungen der Elemisäuren, Helvet. chim. acta, **26**:1659-71, 1943.
 113. SCHAEFFER, J. Zur Phylogese der Talgdrüsen, Ztschr. f. mikr.-anat. Forsch., **22**:579-90, 1930.
 114. SCHMIDT-NIELSEN, K.; SUSKIND, R. R.; and TAYLOR, E. The chemistry of the human sebaceous gland. II. Analysis of human sebaceous material from individual orifices, J. Invest. Dermat., **17**:281-90, 1951.
 115. SCHÖNHEIMER, R.; BEHRING, H. VON; HUMMEL, R.; and SCHINDEL, L. Über die Bedeutung gesättigter Sterine im Organismus, Ztschr. f. physiol. Chem., **192**:73-111, 1930.
 116. SCHULZE, E. Quoted by ROTHMAN and SCHAAF (104) and VELLUZ and LEDERER (130).
 117. SCHULZE, H. Über die Abgrenzung der Sterine gegenüber anderen Alkoholen der Polyterpen-Reihe und über den Bau des Lanosterins und Onocerins, Ztschr. f. physiol. Chem., **238**:35-53, 1936.
 118. SMEDLEY-MACLEAN, I., and HUME, E. M. Fat-deficiency disease of rats; the storage

- of fat in the fat-starved rat, *Biochem. J.*, **35**:990-95, 1941.
119. SOBEL, H. Squalene in sebum and sebum-like materials, *J. Invest. Dermat.*, **13**:333-38, 1949.
 120. STANGER, D. W.; STEINER, P. E.; and BOLYARD, M. N. Unsaponifiable residue of human liver; preparation and primary chromatographic fractionation, *J. Am. Chem. Soc.*, **66**:1621-23, 1944.
 121. SUSKIND, R. R. The chemistry of the human sebaceous gland. I. Histochemical observations, *J. Invest. Dermat.*, **17**:37-54, 1951.
 122. TÄUFEL, K., and HEIMAN, W. Weitere Untersuchungen über die Verbreitung des Squalens in pflanzlichen und tierischen Fetten, *Biochem. Ztschr.*, **306**:123-24, 1940.
 123. TIEDT, J., and TRUTER, E. V. Some normal alcohols occurring in wool wax, *Chemistry & Industry*, pp. 911-12, 1951.
 124. TRUTER, E. V. The constitution of wool wax, *Quart. Rev. (London)*, **5**:390-419, 1951.
 125. TSUJIMOTO, M. A highly unsaturated hydrocarbon in shark liver oil, *Indust. & Engin. Chem.*, **8**:889-96, 1916.
 126. UNNA, P., and GOLODETZ, L. Die Hautfette, *Biochem. Ztschr.*, **20**:469-502, 1909.
 127. ———. Die Cholesterinester der Hornschicht, *ibid.*, **25**:425-26, 1910.
 128. VELICK, S. F. Phytomonic acid, newly branched chain fatty acid, *J. Biol. Chem.*, **152**:533-38, 1944.
 129. ———. Chemistry of *Phytomonas tumefaciens*: concerning structure of phytomonic acid, *ibid.*, **156**:101-7, 1944.
 130. VELLUZ, L., and LEDERER, E. Sur les constituants de la graisse de la laine. I. Essai de mise au point de la question jusqu'à ce jour, *Bull. Soc. chim. biol.*, **27**:211-18, 1945.
 131. VOSER, W.; MIJORIC, M. V.; JEGER, O.; and RUZICKA, L. Über Steroide und Sexualhormone. 186. Über die Konstitution des Lanostadienols (Lanosterins) und seine Zugehörigkeit zu den Steroiden, *Helvet. chim. acta*, **35**:2414-30, 1952.
 132. VOSER, W.; MONTAVON, M.; GÜNTARD, H. H.; JEGER, O.; and RUZICKA, L. Zur Kenntnis der Triterpene; zur Konstitution des Lanostadienols, *Helvet. chim. acta*, **33**:1893-1910, 1950.
 133. WASHBURN, S. L., and LIESE, G. J. The cholesterol content and iodine number of human sebum, *J. Lab. & Clin. Med.*, **41**:199-207, 1953.
 134. WHEATLEY, V. R. The chemical composition of sebum, pp. 90-102. *Livre jubilaire, Société belge de Dermatologie et de Syphiligraphie*. Brussels: Imprimerie médicale et scientifique, 1952.
 135. WEITKAMP, A. W. The acidic constituents of Degras: a new method of structure elucidation, *J. Am. Chem. Soc.*, **67**:447-54, 1945.
 136. WEITKAMP, A. W.; SMILJANIC, A. M.; and ROTHMAN, S. The free fatty acids of human hair fat, *J. Am. Chem. Soc.*, **69**:1936-39, 1947.
 137. WEITZEL, G., and LENNERT, K. Untersuchungen über die Bürzeldrüse der Vögel. I. Die Fettstoffe der Bürzeldrüsen von Enten, *Ztschr. f. physiol. Chem.*, **288**:251-65, 1951.
 138. WIELAND, H., and BENEND, W. Die Beziehungen zwischen Lanosterin und Kryptosterin, *Ztschr. f. physiol. Chem.*, **274**:215-22, 1942.
 139. WIELAND, H., and STANLEY, W. M. Zur Kenntnis der Sterine der Hefe, *Liebigs Ann.*, **489**:31-42, 1931.
 140. WINDAUS, A., and BOCK, F. Über das Provitamin aus dem Sterin der Schweineschwarte, *Ztschr. f. physiol. Chem.*, **245**:168-70, 1937.
 141. WINDAUS, A., and LÜDERS, H. Über die Einwirkung einer alkoholischen Natriumacetatlösung aus Cholesterindibromid, *Ztschr. f. physiol. Chem.*, **109**:183-88, 1920.
 142. ———. Die Einwirkung von Benzoylperoxyd auf Cholesterin, *ibid.*, **115**:257-69, 1921.
 143. WINDAUS, A., and TSCHESCHE, R. Über das sogenannte "Isocholesterin" des Wollfettes, *Ztschr. f. physiol. Chem.*, **190**:51-61, 1930.
 144. WINDAUS, A.; WERDER, F. VON; and GSCHAUER, B. Über die Zahl der Kohlenstoffatome in Molekül des Sitosterins und einiger anderer Sterine, *Ber. deutsch. chem. Gesellsch.*, **65**:1006-9, 1932.
 145. WOODWARD, R. B., and BLOCH, K. The cyclization of squalene in cholesterol synthesis, *J. Am. Chem. Soc.*, **15**:2023, 1953.
 146. ZEHENDER, F. Über den Gehalt an Triglyceriden im menschlichen Hauttalg, *Helvet. chim. acta*, **29**:973-79, 1946.

CHAPTER 14

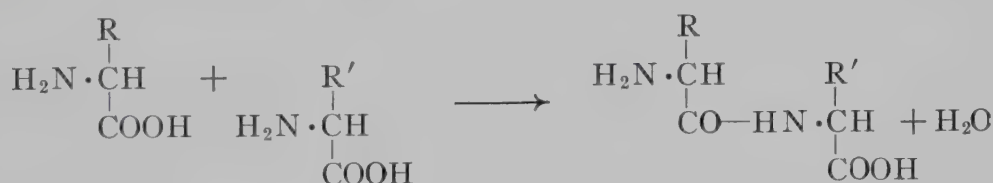
Nitrogenous Constituents; General Data

I. INTRODUCTORY REMARKS ON PROTEINS	337
II. THE TOTAL NITROGEN CONTENT	341
III. THE TOTAL SULFUR CONTENT	341

I. INTRODUCTORY REMARKS ON PROTEINS (8, 20, 1, 12, 2, 3)

CLASSICAL protein chemistry as developed early in this century by Franz Hofmeister (13) and by Emil Fischer (11) has taught us that all proteins are made up by α -amino acids joined together by the elimination of a

water molecule between the $-\text{COOH}$ group of one and the $-\text{NH}_2$ group of another to form a peptide linkage in the following fashion:

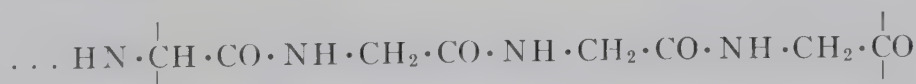


or, in an abbreviated form,



Single amino acids can be coupled in this way to form di-, tri-, and higher peptides. If many amino acids are linked in this fashion,

a peptide or polypeptide chain results. The frame of such a chain is usually written in an abbreviated form as follows:



Peptide linkages can be split by mild acids, alkalies, and proteolytic enzymes into smaller units and finally into single amino acids. The splitting of the peptide linkage is

achieved by uptake of water (hydrolysis). The following amino acids are known to occur in proteins (9):

AMINO ACIDS OBTAINED BY THE HYDROLYSIS OF PROTEINS

A. Acids with equal number of basic and acid groups (one each except No. 17):

1. Glycine (glycocoll): $\text{CH}_2(\text{NH}_2)\text{COOH}$
2. Alanine: $\text{CH}_3\text{CH}(\text{NH}_2)\text{COOH}$
3. Valine: $(\text{CH}_3)_2\text{CHCH}(\text{NH}_2)\text{COOH}$
4. Leucine: $(\text{CH}_3)_2\text{CHCH}_2\text{CH}(\text{NH}_2)\text{COOH}$
5. Norleucine: $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$
6. Isoleucine: $\text{C}_2\text{H}_5\text{CH}(\text{CH}_3)\text{CH}(\text{NH}_2)\text{COOH}$
7. Phenylalanine: $\text{C}_6\text{H}_5\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$
8. Tyrosine: $p\text{-HOC}_6\text{H}_4\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$
9. Threonine: $\text{CH}_3\text{CHOHCH}(\text{NH}_2)\text{COOH}$
10. Serine: $\text{CH}_2\text{OHCH}(\text{NH}_2)\text{COOH}$
11. Proline:

$$\begin{array}{c}
 \text{CH}_2 - \text{CH}_2 \\
 | \quad \quad | \\
 \text{CH}_2 \quad \quad \text{CH} - \text{COOH} \\
 \diagdown \quad \diagup \\
 \text{N} \\
 | \\
 \text{H}
 \end{array}$$
12. Hydroxyproline:

$$\begin{array}{c}
 \text{OH} \\
 | \\
 \text{CH} \\
 | \quad \quad | \\
 \text{CH}_2 \quad \quad \text{CH}_2 \\
 \diagdown \quad \diagup \\
 \text{N} \\
 | \\
 \text{H}
 \end{array}$$
13. Tryptophane:

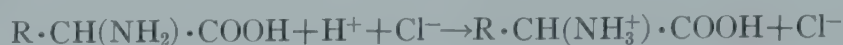
$$\begin{array}{c}
 \text{C} - \text{CH}_2\text{CH}(\text{NH}_2)\text{COOH} \\
 || \\
 \text{CH} \\
 | \\
 \text{N} - \text{H}
 \end{array}$$
14. Thyroxine:

$$\begin{array}{c}
 \text{I} \quad \quad \text{I} \\
 | \quad \quad | \\
 \text{HO} - \text{C}_6\text{H}_3 - \text{O} - \text{C}_6\text{H}_3 - \text{CH}_2\text{CH}(\text{NH}_2)\text{COOH} \\
 | \quad \quad | \\
 \text{I} \quad \quad \text{I}
 \end{array}$$
15. Iodogorgoic acid:

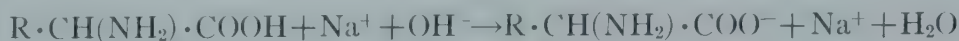
$$\begin{array}{c}
 \text{I} \\
 | \\
 \text{HO} - \text{C}_6\text{H}_4 - \text{CH}_2\text{CH}(\text{NH}_2)\text{COOH} \\
 | \\
 \text{I}
 \end{array}$$
16. Methionine: $\text{CH}_3\text{SCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$
17. Cystine:

$$\begin{array}{c}
 \text{SCH}_2\text{CH}(\text{NH}_2)\text{COOH} \\
 | \\
 \text{SCH}_2\text{CH}(\text{NH}_2)\text{COOH}
 \end{array}$$
18. Cysteine: $\text{HSCH}_2\text{CH}(\text{NH}_2)\text{COOH}$

In acid solutions the amino acids take up the hydrogen ion of the acid to form a cation, which, when subjected to an electrical field, will migrate to the negative pole:



In alkaline solutions amino acids lose a hydrogen ion to the alkali and form anions which migrate to the positive pole:



At a definite hydrogen-ion concentration the net electrical charge is zero; the compound moves neither to the anode nor to the cathode. This hydrogen-ion concentration is called the "isoelectric point."

Like amino acids, most proteins are amphoteric and combine with acids and bases to form water-soluble salts. This is a reversible process as long as the acid or alkali used is not so strong as to cause hydrolysis or denaturation.

The isoelectric points of some proteins lie near neutrality. Others have predominantly acidic or predominantly basic properties, according to the number of free acidic and/or basic groups present. Acidic proteins are more soluble in alkalies, and basic proteins more soluble in acids. The acid- and base-binding capacity of a protein thus depends on its amino acid composition.

Analyses of the amino acid content of different proteins revealed that often the numbers of molecules of different amino acids are in a simple arithmetical ratio to one another. In the globin of hemoglobin, for instance, lysine comprises $\frac{1}{16}$; histidine $\frac{1}{8}$; aspartic acid $\frac{1}{36}$; glutamic acid, tyrosine, proline, and arginine $\frac{1}{48}$; and cysteine $\frac{1}{192}$ of all the constituent amino acids. This means that if we could look along a globin polypeptide chain, we should expect to see every lysine residue separated by 15 other amino acid residues; every histidine residue by 17 other residues; etc. (3).

These findings led Bergmann (2, 3, 4) to assume that in all proteins each amino acid residue recurs with a characteristic whole-number frequency. His calculations indicated that proteins are built of 288 amino acid residues or multiples thereof. While the postulate of equal units had to be abandoned on

the basis of newer analytical data, there can be no doubt that some periodicity does occur in the order of amino acids in proteins, and this is especially true for fibrous proteins.

X-ray diffraction studies (p. 391) have led to the classification of proteins into two distinctly different classes: the "globular" and the "fibrous" proteins. In the former group, to which the plasma proteins and most cell proteins belong, the polypeptide chains are coiled or folded in close sets; in some of them the molecules are folded in such a way that the proteins are almost spherical in shape. In fibrous proteins the polypeptide chains are elongated and form more or less straight lines; to this group belong fibrin, silk protein, collagen, elastin, keratins, and myosin; in these molecules the elongated chains lie parallel to one another in grids and are connected by side chains to one another.

This classification is not identical with the older distinctions of native and water-soluble proteins from the water-insoluble and hard "albuminoids" or "scleroproteins," which have a supporting or protective function in the animal organism. Myosin, for instance, although neither hard nor having a supporting function, belongs to the group of fibrous proteins.

In addition to the simple proteins which, on hydrolysis, yield only amino acids, the conjugated proteins comprise a large group of compounds which represent proteins united with other substances not protein in nature. The most important representatives of this group are hemoprotein, the nucleoproteins, glycoproteins, lipoproteins, phosphoproteins, and chromoproteins. It appears that in living cells the majority of proteins

are conjugated with nonprotein substances. In attempts to isolate proteins from the cytoplasm, the bonds connecting the proteins with other substances are broken rather

easily, and for this reason relatively little is known about cellular proteins as compared with blood proteins, virus proteins, and keratins.

II. THE TOTAL NITROGEN CONTENT

Because of the inhomogeneity of the skin, estimations of its total nitrogen content yield but little information. Collagen, constituting 72–79 per cent of the dry weight of the skin (14, 17), is the most abundantly represented nitrogenous substance, with an average nitrogen content of 18.6 per cent of the dry weight. Eichelberger *et al.* (10) calculated that out of the 16.07 gm. of nitrogen in 100 gm. of dry, fat-free human skin, 11.32 gm., or 70 per cent of the total nitrogen, is present in the form of collagen and elastin.

The fluctuations in the estimated nitrogen content in fresh skin reflect, principally, different amounts of water and fat. Varia-

tions of from 3.5 to 5.4 per cent nitrogen in fresh human skin have been reported (21, 16). The average value for 1 kg. of wet, fat-free human skin has been reported as 45.5 ± 3.8 gm. of nitrogen, of which 33.7 ± 4.5 gm. of nitrogen, or 74 per cent, was collagen-nitrogen (10).

The nitrogen content of the epidermis in the mouse was found to be 4.56 per cent of the wet weight (19), a figure which shows that the nitrogen content of the epidermis is in the same range as that of the total skin.

The nitrogen content of collagen, elastin, and connective-tissue ground substance is discussed in subsequent chapters.

III. THE TOTAL SULFUR CONTENT

Methionine, cysteine, and cystine are the sulfur-containing amino acids of skin proteins. In addition, the mucopolysaccharides of the connective ground substance contain

plained (Fig. 1). The skin of adult men, rabbits (15), and rats (7) contains only 60 per cent as much sulfur as does that of the young. Klauder and Brown's interpretation was

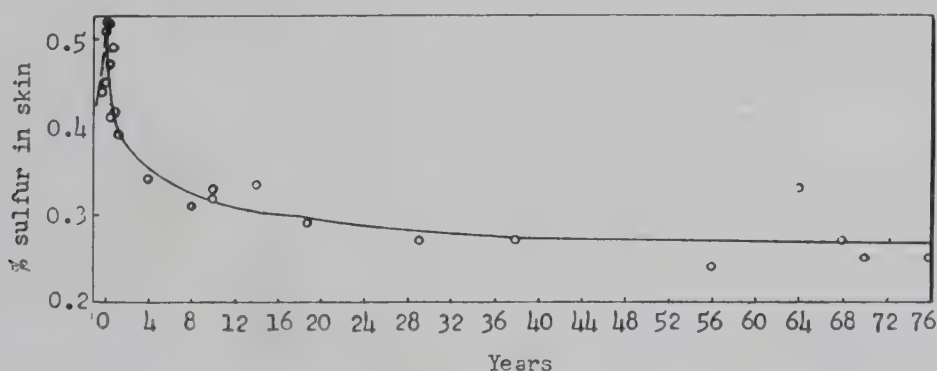


FIG. 1.—Curve showing the percentage per dry weight of total sulfur content in the skin of man at different ages. From Klauder and Brown (15). (Reproduced by permission of Dr. Klauder and the American Medical Association.)

sulfur in the form of chondroitin sulfuric and mucoitin sulfuric acids.

The original findings of Klauder and Brown (15) on the higher sulfur content in infant skin as compared with that of adults in man and animals has remained unex-

plained (Fig. 1). The skin of adult men, rabbits (15), and rats (7) contains only 60 per cent as much sulfur as does that of the young. Klauder and Brown's interpretation was that the difference is due to a higher glutathione content in the skin of the young; but this has not been actually demonstrated. It was assumed that the skin in the adult has a relatively higher concentration of compounds containing the disulfide ($-S-S-$)

linkage of cystine, while the skin of the young has more sulfur in the form of sulfhydryl groups, and that this difference may contribute to the greater strength and rigidity of adult skin (7). However, this idea had to be abandoned when Bonting (7) showed that the percentage of the —S—S— bonds in the total cysteine-cystine fraction is the same in the young as in the adult. Bonting found that with advancing age both the cysteine-cystine-sulfur and the methionine-

sulfur content of the skin, as related to total nitrogen content, drops considerably. Cysteine-cystine-sulfur drops to 44 per cent, and methionine-sulfur to 75 per cent, of the original amount.

There is a possibility that, in addition to the decrease in sulfur-containing amino acids, a decrease in the amount of the connective-tissue ground substance with advancing age (7) contributes to the decrease in sulfur content (pp. 449–50).

BIBLIOGRAPHY

1. BALDWIN, E. Dynamic aspects of biochemistry. London: Cambridge University Press, 1949.
2. BERGMANN, M. Proteins and proteolytic enzymes, Harvey Lect., **31**:37–56, 1931.
3. BERGMANN, M., and NIEMANN, C. On the structure of proteins: cattle hemoglobin, egg albumin, cattle fibrin, gelatin, J. Biol. Chem., **118**:301–14, 1937.
4. ———. On the structure of silk fibroin, *ibid.*, **122**:577–96, 1938.
5. BERGY, C. A. The action of thioglycolates, Am. Prof. Pharmacist, **14**:1014–18, 1056–57, 1948.
6. BLOCK, R. J., and BOLLING, D. The amino acid composition of proteins and foods. Analytical methods and results. 2d ed. Springfield, Ill.: Charles C Thomas, 1951.
7. BONTING, S. J. L., JR. Cysteine, cystine, and methionine in the skin of young and adult rats, Biochim. et biophys. acta, **6**:183–86, 1950.
8. COHN, E. J., and EDSALL, J. T. Proteins, amino acids and peptides as ions and dipolar ions. New York: Reinhold Publishing Corp., 1943.
9. CONANT, J. B., and BLATT, A. H. The chemistry of organic compounds. 3d ed. New York: Macmillan Co., 1948.
10. EISELE, C., and EICHELBERGER, L. Water, electrolyte and nitrogen content of human skin, Proc. Soc. Exper. Biol. & Med., **58**: 97–100, 1945.
11. FISCHER, E. Untersuchungen über Aminosäuren, Polypeptide und Proteine. Berlin: J. Springer, 1906.
12. HAUROWITZ, F. Chemistry and biology of proteins. New York: Academic Press, Inc., 1950.
13. HOFMEISTER, F. Über Bau und Gruppierung der Eiweisskörper, Ergebn. d. Physiol., **1**:759–802, 1902.
14. JACOBS, J. L. A modified technique for determining protein fractions of animal skin, J. Am. Leather Chemists A., **44**:722–37, 1949.
15. KLAUDER, J. V., and BROWN, H. Certain phases of sulfur metabolism of the skin, Arch. Dermat. & Syph., **34**:568–81, 1936.
16. NADEL, A. Chemische Studien an der menschlichen Haut. II. Untersuchungen an der normalen und der pathologischen Menschenhaut, Arch. f. Dermat. u. Syph., **165**:507–24, 1932.
17. NEUMAN, R. E., and LOGAN, M. A. The determination of collagen and elastin in tissues, J. Biol. Chem., **186**:549–56, 1950.
18. ROBERTS, E., and FRANKEL, S. Urea and ammonia content of mouse epidermis, Arch. Biochem., **20**:386–93, 1949.
19. ———. Arginase activity and nitrogen content in epidermal carcinogenesis in mice, Cancer Research, **9**:231–37, 1949.
20. SCHMIDT, C. L. A. The chemistry of the amino acids and proteins. 2d ed. Springfield, Ill.: Charles C Thomas, 1944.
21. URBACH, E. Beiträge zu einer physiologischen und pathologischen Chemie der Haut. II. Der Wasser-, Kochsalz-, Reststickstoff-, und Fettgehalt der Haut in der Norm und unter pathologischen Verhältnissen, Arch. f. Dermat. u. Syph., **156**:73–101, 1928.

CHAPTER 15

Epidermal Proteins

I. THE AMINO ACID COMPOSITION OF EPIDERMAL PROTEINS	343
II. THE PHYSICAL NATURE OF EPIDERMAL PROTEINS	344
III. NONPROTEIN-NITROGEN IN THE EPIDERMIS	347
IV. THE AMINO ACID COMPOSITION OF KERATINS	349
A. General	349
B. Tyrosine	351
C. Sulfur-containing Amino Acids	351
D. Basic Amino Acids	353
E. Dicarboxylic Amino Acids	354
V. THE MOLECULAR STRUCTURE OF KERATINS	354
A. General Structure	354
B. Disulfide Cross-Linkages	356
C. Salt Cross-Linkages	357
D. Hydrogen Bonds	357
VI. ENZYMATIC DIGESTION OF KERATINS	359
VII. CLASSIFICATION OF KERATINS	360

I. THE AMINO ACID COMPOSITION OF EPIDERMAL PROTEINS

A FRAGMENTARY picture of the amino acid composition of epidermal proteins emerges from two separate sources in the literature (Table 1). Unfortunately, two different species were examined in the two publications concerned in Table 1. In spite of the species difference, the cystine, tryptophane, and lysine values are in good agreement. But those for methionine, phenylalanine, and arginine differ greatly.

As compared with the composition of dermal proteins, Mardashev (83) states that epidermal proteins contain three times as much tyrosine, histidine, and cystine and four times as much tryptophane as do the dermal proteins (Table 2). This difference, of course, reflects the great dissimilarity in composition between connective-tissue

fibers and cell proteins, in that collagen and elastin are extremely poor in histidine and lack cystine, tyrosine, and tryptophane entirely or almost entirely.

Concerning methionine values, satisfactory agreement can be calculated for the total skin of rats from the data of Mardashev (83) and Bonting (30): The values for methionine-nitrogen as per cent of protein-nitrogen are 1.26 and 1.16 per cent, respectively, for the adult animal.

The molecular ratio of the three basic amino acids—histidine, lysine, arginine—for epidermis does not show any regularity which would suggest the 1:4:12 relation found in typical hard keratins (24) (p. 353). This is not surprising, in view of the fact that the soft keratins, which derive from

epidermal proteins, do not show this regularity either, or at least not consistently (45). Moreover, the epidermal proteins ap-

parently consist of at least two physically and chemically different types—the fibrous and the nonfibrous proteins.

TABLE 1*
AMINO ACIDS IN EPIDERMIS

Species	Tyrosine	Cystine	Methionine	Tryptophane	Phenylalanine	Histidine	Arginine	Lysine	Leucine	Isoleucine	Threonine	Valine	Glutamic
Mouse (97).....		1.51	0.91	0.98	1.46	3.33	11.50	5.15	4.17	2.65	2.43	2.84	7.90
Human (83)...	4.05	1.42	1.60	1.02	4.78	2.64	6.71	5.92

* Values expressed in percentages of amino-N in total protein.

TABLE 2*
AMINO ACIDS IN HUMAN EPIDERMIS AND CORIUM (83)

	Tyrosine	Cystine	Methionine	Tryptophane	Phenylalanine	Histidine	Arginine	Lysine
Epidermis.....	4.05	1.42	1.6	1.02	4.78	2.64	6.71	5.92
Corium.....	1.27	0.51	1.22	0.24	2.76	0.97	7.16	4.17
Total skin.....		0.65	0.40	1.95	5.15

* Values expressed in percentages of amino-N in total protein.

II. THE PHYSICAL NATURE OF EPIDERMAL PROTEINS

On the basis of microscopic and histochemical evidence, Unna (126) postulated that epidermal cells consist of two physically and chemically different groups of proteins. The first is a fibrillary framework ("spongio-plasma") and the second, a microscopically amorphous material ("granoplasma"), which fills the spaces between the fibrils. The solubility and staining reactions of the two groups of proteins are distinctly different, the solubility of the nonfibrillary substance being much greater. The fibrillary material, usually called "tonofibrils" by morphologists, is birefringent (109). Chemically, these two groups of proteins can be contrasted as fibrous and globular proteins.

In spite of the fabulous development of morphological examination methods (phase microscopy, electron microscopy), the morphology of the tonofibrils, their relation to intercellular spines, their continuity beyond

cell boundaries, their connection with the argyrophil connective-tissue fibers (64), and even their very existence are not established on morphological grounds (96, 2, 76, 62). X-ray diffraction studies, however, leave no doubt that the noncornified epidermis does contain fibrils which have the same "α-keratin" spectrogram (p. 354) as do keratin fibers (42, 43, 59, 11, 13, 107). This, of course, does not mean that the epidermal fibrils are actually identical with keratin fibrils, but only that they have a similar fibrillary structure. Similar fibers are found also in epithelia of noncornifying endodermal linings (105).

The view that the epidermal fibrils cross the cell boundaries and form a continuous network (94) implies that the intercellular bridges of the Malpighian layer are parts of the same fiber system (Fig. 1). In the early twenties the rather revolutionary theory

was developed that the continuous epidermal fibril system derives from and is continuous with the connective-tissue fibril system. According to Frieboes (54), the mesodermal fiber system represents a supporting structure for the protoplasmic mass of the epidermis, in which the nuclei are about evenly distributed but which does not consist of single individual cells.

While this theory has not received acceptance, micromanipulator experiments of

ing with hot water, while keratin fibers undergo such a transformation only if stretched in hot water. The molecular chains of fibrils in the epidermis have no orientation with respect to an axis in the plane of the surface, whereas fibrils of fully keratinized fibers are parallel to such an axis. The epidermal fibrils display the phenomenon of thermal supercontraction (p. 355), like myosin fibers of muscle and unlike keratin fibers. After treatment with hot water the epidermal

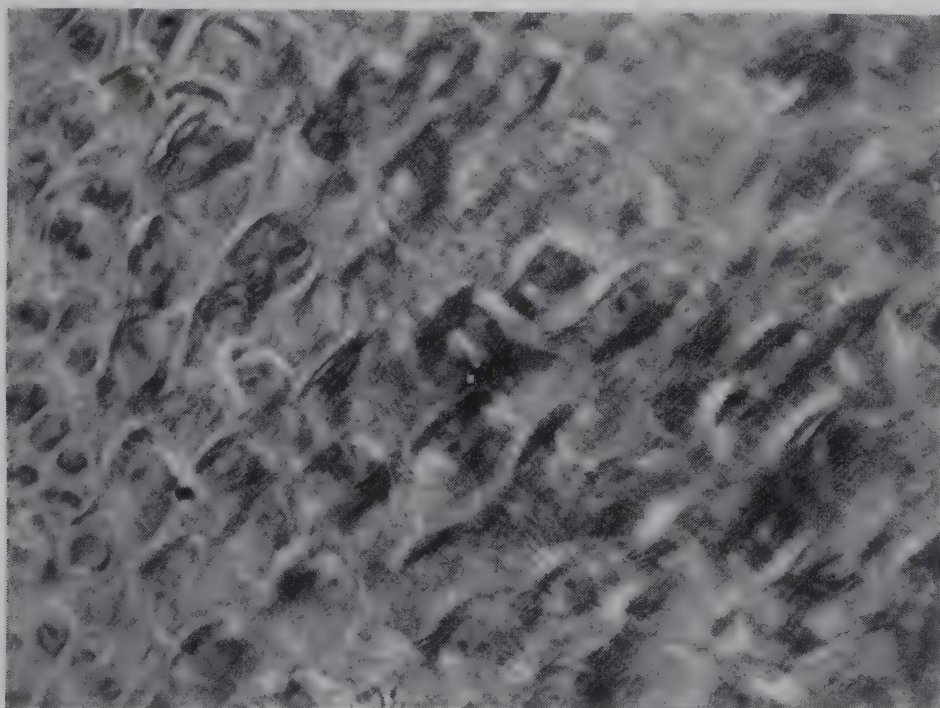


FIG. 1.—Epidermal tonofibrils and spines. Photo taken in polarized light. (Courtesy of Dr. John Facq, Toni Laboratories, Chicago.)

Chambers and Renyi (37) indicated that isolated damage to a single cell, with disruption of its intercellular bridges, involves degeneration and death of neighboring cells—an observation which seems to indicate that the integrity of the intercellular bridges is essential for the life of the cells. Possibly this is so because the bridges continue into the cell and play a role in the transportation of nutrient material.

Differences in the behavior of epidermal and keratin fibrils were studied in detail by Rudall (105, 107). The spectrogram of epidermal fibrils can be transformed from the α - into the β -form (p. 354) by simple heat-

fibrils, like myosin and fibrin, display a special type of X-ray spectrogram, the so-called “cross- β -form,” which indicates a special type of loosening of intramolecular bonds. In this loosened form the fibers contract when treated with strong urea solutions.

The first attempt to separate the fibrous proteins out of the epidermal cells was made by Rudall (105, 106, 107). He treated the stratum mucosum with a 50 per cent urea solution, which dissolved almost all the cytoplasm but left the nuclei intact. The resulting viscous solution was subjected to repeated centrifugation and filtration. The clear solution finally obtained could be pre-

cipitated with ammonium sulfate and dried as a film. When stretched before drying, this material yielded oriented fibers with all the physical properties of the epidermal fibers *in situ*. Rudall called this material *epidermin*. The significance of this work for dermatological research is obvious.

Rudall (107) has recently reported in detail the further development of this work. He divided the epidermis of the cow's hairless nose into three layers, the first containing the stratum corneum and the outermost

responding layers, the difference being greatest in the stratum corneum and least in the innermost layer. The fibrous proteins represented the major portion of total epidermal proteins.

Rudall has also demonstrated that the internal stability of the fiber structures increases progressively as one goes from the inner layers outward. This was shown by thermal contraction curves (Fig. 2). Similarly, the α -pattern of fibers in the lower parts of the epidermis changed into the β -

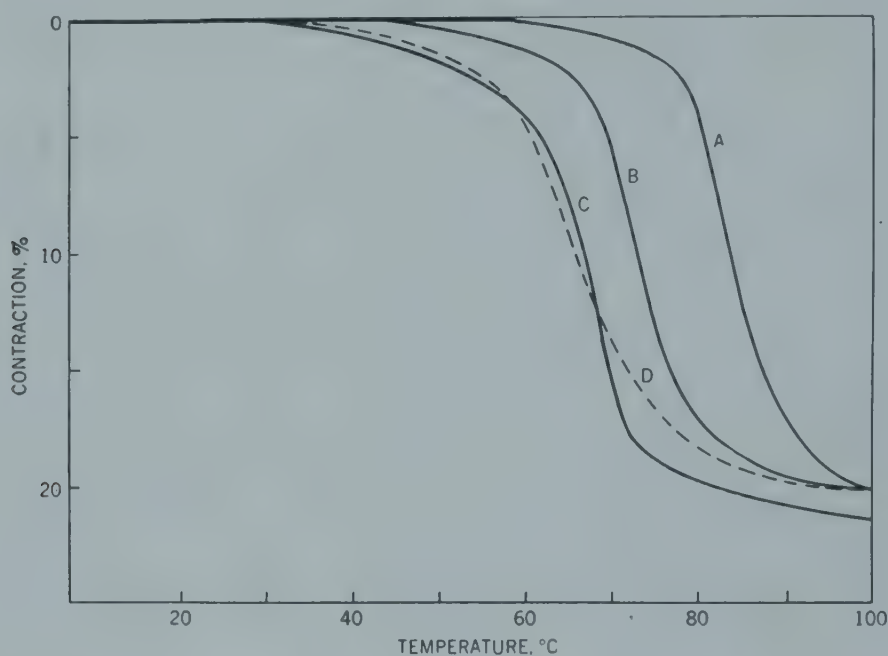


FIG. 2.—Thermal contraction curves of various layers of the epidermis: *A*, stratum corneum; *B*, outer mucosum; *C*, inner mucosum. Curve for film of epidermal protein, precipitated from urea solution, is shown at *D*. From Rudall (107). (Reproduced by permission of the author and Academic Press, Inc.)

cell layers of the rete; the second, the rest of the outer half; and the third, the inner half of the mucous layer. In all three layers he was able to separate two water-soluble proteins by dissolving in urea solution, dialysis, and acid precipitation. One, the fibrous protein or epidermin, having an α -keratin pattern, precipitated from neutral solutions at pH 5.5. The other, a nonfibrous protein, precipitated at pH 4.5 and had a β -pattern. However, this β -pattern showed no orientation in reference to the plane of the film. Surprisingly, the nonfibrous proteins of all layers had a considerably higher sulfur content than did the fibrous proteins of the cor-

pattern with greater ease, i.e., at much lower temperatures, in water than did the fibers of the horny layer.

Rudall found a close similarity between the epidermal fibrils and the fibrinogen-fibrin group of proteins, particularly in their solubility in urea and in urea-salt solutions. From the basal to the horny layers the solubility in urea decreased in much the same way as the solubility of fibrinogen decreases on conversion to fibrin.

Like keratin fibers, epidermal fibers could be easily stretched up to 200–300 per cent of the original length. Stretching was best achieved in saturated ammonium sulfate

solutions at high temperatures. Stretching of epidermal fibers, like that of keratin fibers, was reversible. The cross- β -pattern could be reversed into the α -pattern. The molecular weight of the epidermal protein which was originally dissolved in urea solution was estimated by the ultracentrifugation method and was found to be in the range of 60,000.

III. NONPROTEIN-NITROGEN IN THE EPIDERMIS

In living cells, hydrolytic and oxidative decomposition of proteins and their resynthesis are going on continuously. The ratio of protein decomposition products to the total nitrogen content, at any minute, reflects only a momentary situation. Still this ratio may be regarded, to some extent, as a measure of the catabolic part of protein metabolism.

Older data (127, 90) indicated that 17–19 per cent of the total nitrogen in total skin is nonprotein-nitrogen. Also it was claimed that this ratio is increased in some diseased tissues—for instance, lupus vulgaris lesions (90)—but not in others—for instance, psoriasis—possibly because of a greater tendency to rapid protein synthesis (100). It was found (127) that the nonprotein-nitrogen of the skin is greatly increased in kidney insufficiency because of insufficient elimination of protein metabolism waste products (127).

The average nonprotein-nitrogen of the mouse epidermis was estimated to be 17.4 per cent of its total nitrogen content by Roberts and Frankel (99). Thus, if it is permissible to compare data on mouse skin and human skin, it appears that the nonprotein-nitrogen content of the total skin is in the same range as that of the epidermis, an unexpected finding in view of the higher metabolic rate of the epidermis.

The most abundant compounds in the nonprotein-nitrogen fraction of the epidermis are amino acids, urea, and ammonia. The free amino acids extracted from mouse epidermis (and epithelial tumors) (100) are diagrammatically given in Figure 3.¹ These

The extraction of globular proteins from total skin can be achieved by extraction with salt solutions, according to Roddy (101). A technic to estimate the amount of globular proteins in the skin (by precipitation with 3 per cent trichloroacetic acid) was recently described by Jacobs (72). This author found that the amount of globular proteins in the skin decreases with the age of the animal.

data will be valuable in the study of the biochemistry of keratinization. While the sulfur-containing amino acid methionine and the tripeptide glutathione are among the freely occurring low-molecular nitrogenous

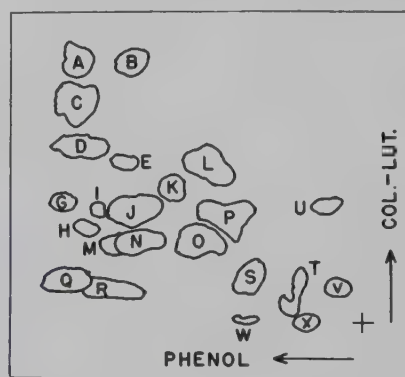


FIG. 3.—Diagram of constituents of paper chromatograms made from epidermal and tumor extracts: A, phenylalanine; B, tyrosine; C, leucine; D, valine; E, methionine sulfone; G, proline; H, histidine; I, hydroxyproline; J, alanine; K, threonine; L, taurine; M, β -alanine or citrulline; N, glutamine or serylglycylglycine; O, glycine; P, serine; Q, arginine; R, lysine; S, glutamic acid; T, aspartic acid; U, cysteic acid; V, "oxidized" glutathione; W, "underglutamic acid," unidentified; X, glutathione. Treatment of the extracts with H_2O_2 has converted methionine to methionine sulfone and cystine to cysteic acid. From Roberts and Tishkott (100). (Reproduced by permission of Dr. Roberts and the American Association for the Advancement of Science.)

constituents which can be extracted from the epidermis with aqueous solvents, sulfur-containing free amino acids or peptides have not been found in aqueous extracts of kera-

1. For free amino acids in human epidermis see E. V. Cowdry, Epidermal carcinogenesis, *Advances in Cancer Research*, 1:57–101, 1953.

tinized products, such as hair (29). This would indicate that in the keratinization process all or most of the sulfur-containing amino acids are built into the keratin molecules, while other amino acids, such as glutamic acid, valine, and leucine (29), are shed when keratinization takes place (p. 372). Otherwise, the available data on the occurrence of free amino acids do not reveal any specific feature of nitrogen metabolism in the epidermis.

However, a highly characteristic feature of the epidermis is its high urea and ammonia content. According to Roberts and

acids (see below). The data on the urea content point to an unusually high arginase activity of the epidermis. That such high activity actually exists was directly demonstrated for mouse epidermis by Greenstein (63) and by Roberts and Frankel (99); for human epidermis by Mardashev and Semina (84) and by Van Scott (128). The latter has shown that the high arginase activity is a function of the Malpighian layer and possibly of the granular layer. So far, the rete cells seem to have the highest arginase activity of all tissues (pp. 204 and 574).² The enormous arginase content of squamous-cell carcinomas (83, 99) can thus be linked to the uninhibited proliferation of Malpighian cells. Interestingly, Mardashev and Semina (84) also found increased arginase activity of the epidermis in animals which were kept on a low-protein diet.

TABLE 3*
UREA AND AMMONIA CONTENT
OF DIFFERENT TISSUES (98)

MATERIAL	No. OF SAM- PLES	UREA-N		AMMONIA-N	
		Mean	Range	Mean	Range
Human epidermis (from breast).....	6	33	17-64	24	17-46
Mouse epidermis.....	20	77	55-98	24	16-31
Mouse kidney.....	3	35	30-39	17	14-20
Mouse liver.....	7	21	15-30	11	7-15
Mouse blood..	10	16	13-21

* Values expressed in mg N/100 gm fresh tissue and for blood in mg N/100 ml.

Frankel (98), the epidermis is the tissue much the richest in urea and ammonia in the mouse (Table 3). The values in human epidermis are somewhat lower but still unusually high. In the mouse the urea and ammonia content of the epidermis increases from the prenatal period up to a few days after birth (between 5 and 11 days) and then declines, but still remains very high throughout life (Table 4).

On the whole, urea-nitrogen and ammonia-nitrogen change in a parallel fashion. The parallelism, however, is certainly not quite regular. This is not surprising, considering that urea is probably a split product in the arginine-ornithine cycle (p. 208), while ammonia derives mainly from oxidative deamination of a great number of amino

TABLE 4*
CHANGES IN UREA AND AMMONIA CONTENT
OF MOUSE EPIDERMIS WITH AGE (98)

AGE	No. OF MICE	UREA-N IN		AMMONIA-N IN	
		Epi- dermis	Der- mis	Epi- dermis	Der- mis
9-12 months..	3	45	24	22	19
9-12 months..	3	87	24	24	20
9-12 months..	3	77	20	21	17
7 days.....	2	111	28	95	29
7 days.....	2	131	40	100	20

* The epidermis was separated from the corium by heat. The skin of adult animals was shaved. Values are expressed in mg N/100 gm fresh tissue.

In addition to splitting into ornithine and urea, arginine is also decomposed by oxidative deamination in the skin of mammals (31) and of birds (123).

In a long series of experiments, Borghi and his associates (31) demonstrated the oxidative deamination by the skin of the following amino acids: arginine, histidine, leucine, cystine, tyrosine, proline, hydroxy-

2. For this and related subjects see also the most recent review article, C. Carruthers and V. Sontzeff, *Biochemistry and physiology of the epidermis*, *Physiol. Rev.*, **33**:229-43, 1953.

proline, and tryptophane. All these amino acids are decomposed if added to skin slices, and the decomposition is accompanied by an increased oxygen uptake and liberation of ammonia in the proportions expected from oxidative deamination.

Whether the arginine-ornithine cycle is operating in epidermal protein metabolism still remains to be demonstrated directly. This probably could be done most conclusively by radioisotope experiments. Should it be demonstrated that the cycle actually operates, this finding would constitute an

important advance in our knowledge of the metabolism of the skin.

Many quantitative experiments will have to be performed, preferably on isolated surviving perfused skin and preferably with isotope materials, before definite conclusions can be drawn on the intermediary nitrogen metabolism of the skin. The interpretation of the available analytical data in terms of metabolic pathways has an uncertainty which is still very great. This holds true also for intermediary lipid and carbohydrate metabolism.

IV. THE AMINO ACID COMPOSITION OF KERATINS

A. GENERAL

In discussing the chemical constituents of keratins, two facts should be kept in mind. First, the term "keratins" refers to materials which have been obtained from keratinous structures (such as hair, nails, horny layers) by more or less thorough purification. It is regrettable that the terms "keratin" and "keratinous structures" are often used rather loosely, as if they were synonymous, so that one may read of "keratins" which contain such and such an amount of ash or fat or water. Keratins are modified proteins, and there are no data to show that all the other constituents of keratinous structures are bound chemically to these modified proteins; they are not part of the keratin molecule.

Second, keratins are a very large group of compounds. In contrast to those proteins which in the crystalline state have a constant composition, such as egg albumin, crystallized enzymes, insulin, etc., keratins show an extremely inconstant amino acid composition, varying not only with preparations from different sources but also in keratins derived from the same type of material. The probable reason for the variable composition is that keratins are products formed from other proteins and that the transformation, which is called the "keratinization process," is by no means uniform. It differs according to the nature of the cellular proteins from which the keratins de-

rive; and even those from the same kind of germinative epithelial cells may show different amino acid composition, according to the velocity of keratinization, which, in turn, may depend on such physiological variants as the nutritional supply to the epithelium or on environmental factors, such as temperature, solar radiation, etc.

The different biological potentialities of epithelial cells which undergo keratinization are best illustrated by the development of hair keratins. This apparently single keratinous structure contains at least four keratins which are different in chemical composition—keratins of the outer and of the inner layer of the cuticle, the keratin of the cortex, and the keratin of the medulla. These different keratins develop from germinative cells which are differently situated topographically and which are not interchangeable. The type of keratin which will be produced from each (concentrically situated) cell layer is genetically predetermined (Figs. 4 and 5).

In the case of sheep wool, the cuticle-keratins can be separated from other parts of the hair by reduction with thioglycolic acid and by alkylolation with ethyl bromide. After this preliminary treatment, the wool is attacked by pepsin in such a way that the cuticle material remains intact, while the interior of the hair is completely dissolved. After this separation, it can be shown that the more resistant cuticle-keratin has a higher sulfur content than the more digest-

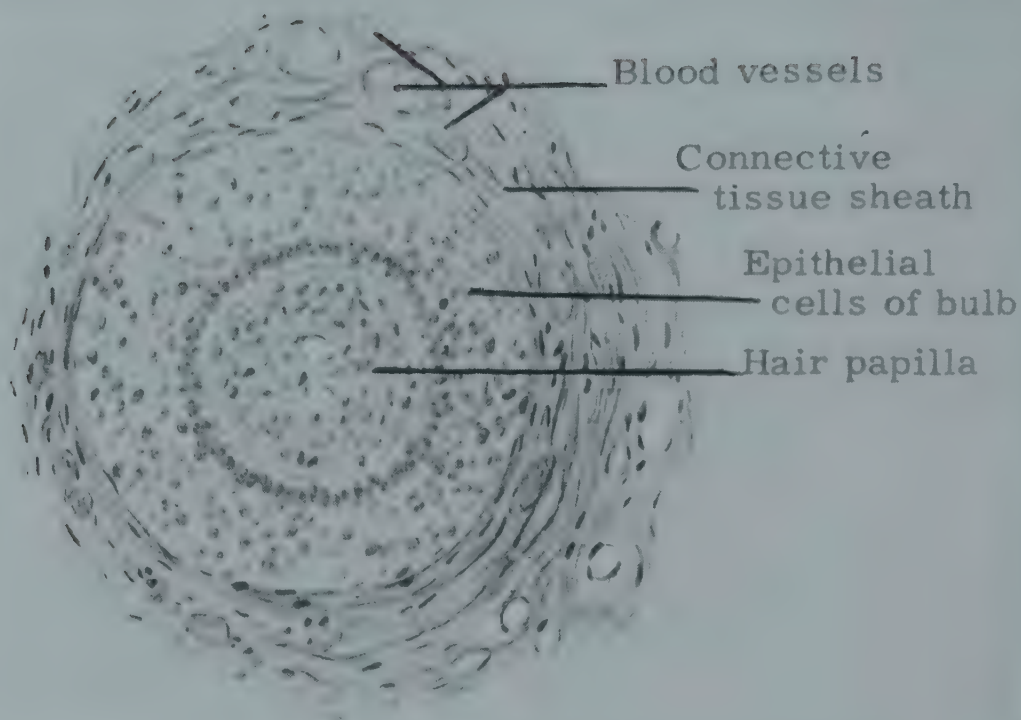


FIG. 4.—Cross-section through hair bulb. From Kopsch (75). (Reproduced by permission of the author and Georg Thieme Verlag.)

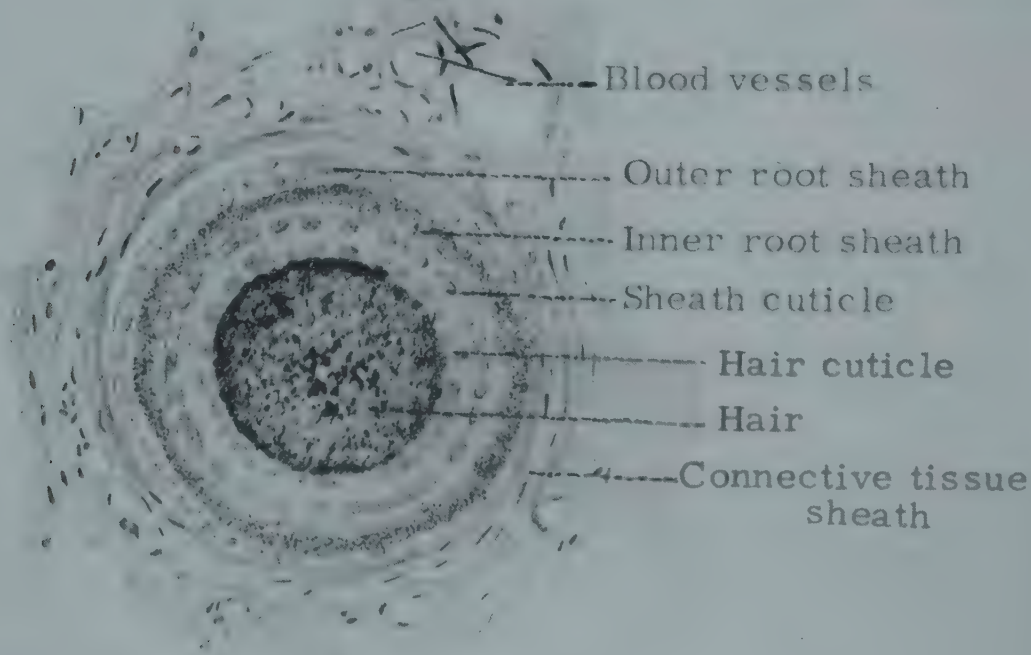


FIG. 5.—Cross-section of hair follicle just above papilla. From Kopsch (75). (Reproduced by permission of the author and Georg Thieme Verlag.)

ible medulla-keratin and cortex-keratin (55). There is evidence that cuticle and cortex are connected with each other in the hair by an intercellular connecting substance, which is removed by tryptic digestion. Thus it can be said that the two keratins are two distinct individual compounds, and not artificial degradation products of a larger keratin complex which might be formed during separation. It has actually been shown that separation of the two layers can be achieved without any appreciable chemical decomposition; on the contrary, in greatly swollen wool fibers, the cuticle is, as a matter of fact, already separated from the cortex (50). This is no surprise to the histologist, who is able to differentiate the two layers by their morphologically different appearance under the conventional microscope.

With conventional microscopy, the flattened cells forming the imbricated scaly cuticle of the hair appear as a homogeneous structure. But with the electron microscope (88), two layers can be distinguished in this sheath: An outer layer, which is smooth and featureless and can be easily removed by tryptic digestion and which is possibly identical with the intercellular cement, and an inner layer, which has a characteristic uneven, pitted surface with ratchet-like edges and which remains untouched by tryptic digestion.

Finally, the medulla of hair can be differentiated easily from the other hair keratins by greater digestibility with tryptic enzymes (52).

Stoves (119) examined histochemically a cross-section of hairs from a species of rabbit, with well-developed and differentiated cuticle, cortex, and medulla. He found, by means of the Sullivan test, that in this hair species the cortex is richer in cystine than the medulla, and therefore the cortex reacts more readily with sodium sulfide. Whereas the cortex dissolves in this reagent, the cuticle merely starts to swell, and the medulla is intact. Considerably more tyrosine is histochemically demonstrable in the medulla than in the other layers.

The relative proportion of different kera-

tins in different species is decisive also for the physical behavior of hair. It appears that permanent setting, for instance, is partly dependent on the quantitative relationship of cuticle to cortex. These few data on the differences in keratins forming the hair clearly indicate that no constant chemical composition of keratins can be expected.

A total of eighteen amino acids occurring in keratins has been demonstrated (86). In contrast to the fibrous proteins in connective tissue—collagen and elastin—keratins do contain all essential amino acids, though some of them are present only in traces, and therefore keratin hydrolysates cannot substitute for other proteins in nutrition.

A feature which is possibly important is the absence of hydroxyproline from human hair (21). It was pointed out (102) that, because hydroxyproline has a fungistatic action, its absence from keratins may constitute a reasonable explanation of the fact that pathogenic hyphomycetes are obligate parasites of keratinized materials and do not enter living cells.

B. TYROSINE

It was claimed by Unna (126) that tyrosine is present in a higher percentage in horny material than it is in cellular proteins. Actually, it appears that the tyrosine content in keratins of horny layer, hair, and nails is extremely variable (25) and that no general rule concerning the tyrosine content of keratins can be formulated (Table 5). In contrast to Unna's contention, Stoves (121) found, histochemically, more tyrosine in the less keratinized medulla than in the cortex and cuticle. Conversely, however, in human epidermis, when Millon's reagent is used for the demonstration of tyrosine, the horny layer appears to be much richer in tyrosine than does the noncornified epidermis. The tyrosine content seems to be particularly high in places with well-developed horny layer, such as the plantar skin (74).

C. SULFUR-CONTAINING AMINO ACIDS

Probably there is only one constant chemical feature which characterizes the

hydrolytic products of keratins as contrasted with all other proteins, namely, a high cystine content. The cystine content seems to parallel the degree of keratinization: the harder and the more enzyme-resistant the keratinous structure, the higher is its cystine content. For instance, human hair contains 15.5 per cent (Table 5), fingernails 12.0 per cent (Table 5), and horny-layer keratin only 3.8 per cent (45) cystine. Also it appears that, with increasing cystine content, the amount of cysteine and methionine decreases. Methionine and cysteine are present in hairs and nails only in traces, but in quite

cystine relation is about 1:15 or even less (81).

Whenever there is reason to believe that keratinization has been incomplete, the keratinized structures contain relatively less cystine and more cysteine and/or methionine.³ The higher cystine content of hair cuticle as compared with that of the cortex has already been mentioned (55). Hess (69) found that, on the average, the cystine in poorly keratinizing nails of arthritics is 18 per cent less than that in nails of normals. Mescon and Flesch (89) found in parakeratotic horny layers a histochemical -SH re-

TABLE 5*
AMINO ACIDS IN KERATINIZED STRUCTURES (25)

	Nitrogen	Sulfur	Histidine	Lysine	Arginine	Cystine	Tyrosine	Tryptophane	Phenylalanine	Glycine
Human hair.....	15.4	5.0	0.6	2.5	8.0	15.5	3.0	0.7	2.6	4.3
Chimpanzee hair....	16.7	4.3	.6	2.0	8.1	15.5	3.3	1.4
Goat hair.....	16.2	3.1	.7	3.2	8.1	8.9	3.0	0.9	4.6	6.3
Cow hair.....	15.3	3.7	.7	2.0	7.5	13.4	3.3	1.4	3.9	10.3
Lamb wool.....	15.4	3.6	.7	2.5	8.7	13.1	4.5	0.7	4.0	6.5
Camel wool.....	15.1	3.1	.6	2.7	8.6	11.0	3.1	0.8	4.1	9.2
Cattle horn.....	16.1	2.6	.6	2.4	8.6	8.2	3.7	0.7	4.0	9.8
Rhinoceros horn....	15.6	2.3	.6	2.6	8.2	8.7	8.6	1.7	5.0	7.4
Fingernails.....	14.9	3.8	.5	2.6	8.5	12.0	3.0	1.1	2.5
Porcupine quills....	15.8	3.0	.6	2.6	7.6	9.4	3.3	0.9	3.6	5.7
Echinus quills.....	15.2	3.8	.5	1.8	6.8	11.9	9.1	2.2	6.8
Hen feathers.....	15.5	2.3	.3	1.6	6.0	6.8	2.2	0.7	5.3	9.5
Snake skins.....	15.2	2.2	.4	1.9	5.4	6.6	5.2	0.9	3.9	13.1
Egg-shell membranes	16.6	3.8	0.9	3.7	9.9	12.7	2.5	2.6	2.0

* Values are expressed in weight percentages. The cystine values have been calculated as cysteine.

substantial amounts in scales of the epidermis (132). If the -SH groups of cysteine are demonstrated histochemically, it looks as though these groups were entirely or almost entirely absent from keratinized structures (57, 89). This is, however, not the case. Methionine was found to occur in such well-keratinized structures as wool (0.5 per cent) (15) and even in steer horn (0.02 per cent) (1). The point, however, is that the relation of nondisulfide-sulfur to disulfide-sulfur becomes smaller as keratinization becomes more complete. While in human horny scales, according to Wilkerson (132), there are about equal amounts of methionine and cystine, in human hair the methionine-

action which was sometimes pronounced, whereas in the normal horny layer this reaction was minimal or absent.

To what degree the horny material is able to accumulate cystine is well illustrated by the data of Marston (85): in plant leaf proteins, the chief dietary source of proteins for sheep, cystine-nitrogen, is 1.2-1.7 per

3. Parakeratotic scales of psoriasis patients have an unusually high -SH content (M. Zingsheim, Die Rolle freier Sulfhydrylgruppen bei der Schuppenflechte, Deutsche med. Wchnschr., 77:1630-31, 1952). Physiologically, the parakeratotic horny layer of certain mucous membranes is also rich in -SH groups (A. Z. Eisen, W. Montagna, and H. B. Chase, Sulfhydryl groups in the skin of the mouse and guinea pig, J. Nat. Cancer Inst., 14:341-53, 1953).

cent of the total protein-nitrogen, while in wool this percentage is 9.

D. BASIC AMINO ACIDS

Keratins of fully keratinized structures contain histidine, lysine, and arginine in quantities corresponding to an average molecular ratio for these amino acids of approximately 1:4:12 (28, 23, 26). This discovery of R. J. Block led to the theory that these basic amino acids are the core of all proteins, that they are of primary importance in the genetic and embryonic development of tissue proteins as they exist in protoplasm, and that they have a directive influence on the general structure of proteins. It was proposed that tissue proteins be classified according to their content in arginine, histidine, and lysine (26).

TABLE 6*

ANALYSIS OF SCALES FROM EXFOLIATIVE
DERMATITIS AND OF NORMAL HORNY
LAYER OF SOLES

	Eckstein (45)	Wilkerson (131)	Block (22)
Humin-N.....	2.02	2.11
Amide-N.....	7.68	3.60
Arginine.....	5.91	10.01	6.00
Lysine.....	4.68	3.06	4.50
Histidine.....	0.64	0.59	0.82
Cystine.....	3.82	2.31	3.40
Tyrosine.....	3.42	5.70
Tryptophane..	1.80	1.49

* Amino acid concentration expressed in percentages of amino-N in total pure keratin-N.

Indeed, the approximation of this regularity can be discovered in hairs of all mammalian species, in bird feathers, in porcupine quills, in horns of cattle and rhinoceros, in fingernails of man, and even in egg-shell membranes, the "keratin" of which belongs to the group of ovokeratins. It was pointed out also that this relatively constant relationship is in contrast to the great variability in the amount of other amino acids, particularly cystine, in keratins (see Table 5). While the constant basic amino acid relationship holds for fully keratinized struc-

tures, it is not present in the keratins of the epidermal horny layer (Table 6). Unfortunately, the available data refer both to the normal horny layer of the soles and to scales from patients with exfoliative dermatitides, and in the publications no distinction is made between these two sources. It is clear, at least to dermatologists, that these two kinds of horny material should have been examined separately, because it is most

TABLE 7

NITROGEN DISTRIBUTION IN
HAIR HYDROLYSATE (20)
(Total N in Hair Keratins,
16.04 Per Cent)

	PER CENT OF TOTAL N	
	Observed	Corrected*
Melanin-N.....	0.56	0.56
Amide-N.....	7.22	7.22
Basic N.....	33.40	35.8
Dicarboxylic N....	11.50	11.5
Monoamino acid-N plus nonamino-N	46.32	44.9

*Corrected for solubility of phosphotungstate.

probable that in exfoliative dermatitis the keratinization is less complete than in the horny layer of normal epidermis. In any case, the conclusion was drawn (22) that epidermal keratins may show incomplete keratinization, as indicated by an irregular molar relationship of histidine:lysine:arginine, this being 1:6:7 in the analyses of Block (22) and of Eckstein (45). But it was also assumed that complete keratinization may occur in the epidermis, because the analyses of Wilkerson (131) showed a 1:6:15 molecular ratio of the three amino acids.

There is no conclusive evidence that arginine is accumulated during keratinization. The total human epidermis contains 6.7 per cent arginine (83) (Table 1, p. 344); and in scales about 6.0 per cent arginine was found by two authors (Table 6). The high arginine content found by Wilkerson (Table 6) might have been due to some differences

in methods (22). Still the fact remains that in hair and nails the arginine content is considerable.

E. DICARBOXYLIC AMINO ACIDS

Glutamic and aspartic acids are present in keratins both in the form of free acids and in the form of amides (p. 339). In human hair, for instance, the dicarboxylic acid amino-nitrogen amounts to 11.5 per cent and the amide-nitrogen to 7.22 per cent of the total keratin-nitrogen (20) (Table 7). Whether the presence of amides has any biological significance has not been investigated. Because

hyphomycetes thrive exceedingly well on glutamine, the occurrence of amides on the surface might be significant.

The total concentration of glutamic acid in human hair keratin was found to be 10.6 per cent; that of aspartic acid, 3.5 per cent (20). It seems that hydroxyglutamic acid is also present in about 1 per cent concentration (20).

Although chemical analyses have been contradictory, Speakman insisted, on the basis of salt-linkage studies, that free acid and free basic side chains are equivalent in wool keratin (116).

V. THE MOLECULAR STRUCTURE OF KERATINS

A. GENERAL STRUCTURE

The basic structure of keratins was elucidated in the 1930's, particularly by the admirable work of W. T. Astbury and J. B. Speakman.

Keratins are fibrous proteins, distinguished by their great resistance to peptic

odidity of amino acid groups (86). As in other fibrous proteins, the shape of the molecules reflects that of macroscopic fibers, the molecules having an elongated structure which is a hundred or more times longer than it is thick. The long axes of the molecule lie roughly parallel to one another; and in the case of hair they lie in the long axis of the macroscopic fiber. In nails the direction of the fibers is transverse (43); and in the horny layer of the epidermis it is quite irregular (42, 43). The long axes consist of polypeptide chains, apparently with a periodicity of three amino acid residues (8).

The parallel polypeptide chains have side branches, and many such side branches of neighboring chains are connected with one another, forming bridges between parallel chains. This structure is usually compared to a tridimensional ladder with rungs connecting the sides at roughly equal distances from one another.

According to Astbury, 50 per cent of the weight of wool is represented by side chains (10). In the normal state of most keratins, the polypeptide chains are folded in a plane transverse to the cross-bridges, and in this state they yield the α -keratin X-ray spectrogram of Astbury. However, the folded chains can be stretched reversibly, and in the stretched state the X-ray spectrogram of β -keratin is obtained (8, 12) (Fig. 6).

According to recent findings, the keratin

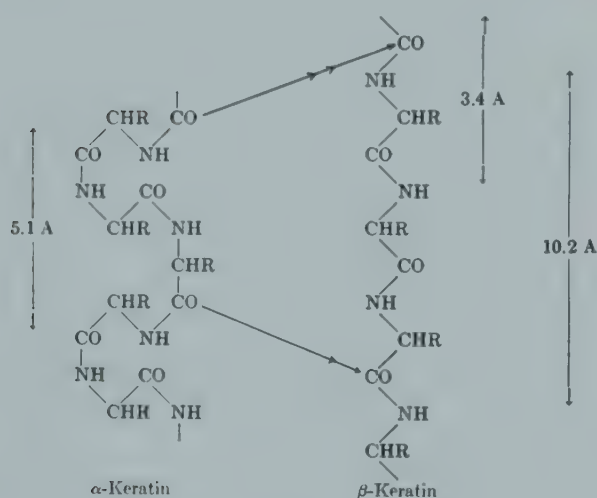


FIG. 6

and tryptic digestion and to hydrolysis by dilute acid and alkalis. They are insoluble in water, salt solutions, and organic solvents. Keratins, like other fibrous proteins, are crystalline structures, inasmuch as their atoms and atomic groups are arranged in a regular manner with a regular repetitive pattern. However, there is no regular peri-

of hair cuticle has the β -configuration in its natural state (6). In some interpretations (129) the α -folds are closed entirely to rings; but most investigators agree that there is no complete ring closure.

If stretched, the keratin fibers tend to regain the original folded state. They have a long-range elasticity with a good ability to recover from deformation (8, 65). It is of the utmost significance for the physiology and pathology of the skin that this elasticity is greatly increased by the presence of water. Pliability is also greatly increased by the uptake of water. Dry keratin fibers taking up water swell predominantly in the transverse direction. When swelling in water is complete, the fibers become 18 per cent thicker in diameter, while their length increases only 1 per cent. This is because water primarily spreads over the surfaces of the polypeptide chain crystallites, which are much longer than they are thick. The thinner the crystallite unit, the greater is the percentage of lateral swelling (8). In the dry state the building stones of the polypeptide chains exert their full cohesive forces on one another. The cohesive forces resist forces tending to extend the folded chain. Or, if the force is too great, the fibers break. When water enters the spaces between the chains, it neutralizes some of these cohesive forces and acts as a lubricant. This mechanism renders it easier to stretch keratin fibers in a wet state than when dry (p. 379).

Although the manner in which water is held firmly and loosely by fibrous structures appears complex (see also p. 499), involving many kinds of chemical and physical forces (34, 56), it appears that the original micelle theory of Naegeli, postulating the existence of submicroscopic elongated bodies which are surrounded, but in the main not penetrated, by liquid, still holds.

An important factor in the elasticity of keratin fibers is the existence of disulfide cross-bridges (65, 67, 114), which act like a cement between the micelles (6). Harris (67) showed that if the disulfide cross-links (p. 356) are ruptured, the elastic modulus of keratin fibers in water is reduced to less than

one-tenth its original value. Spontaneous contraction after stretching is mainly a function of the cross-linkages, which tend to settle in the "most comfortable position" (8) dictated by attractive forces. They are best satisfied when the main chains are folded, as is the case in their natural state. Hence the tendency to spontaneous contraction after stretching. The role of salt linkages in determining physical behavior was studied by Speakman (114, 115).

The effects of steam, alkalies, acids, and a number of reducing, oxidizing, and deaminating chemicals on keratin fibers were studied in great detail. Essentially, their effects consist of loosening or breaking linkages within the main chains, within cross-

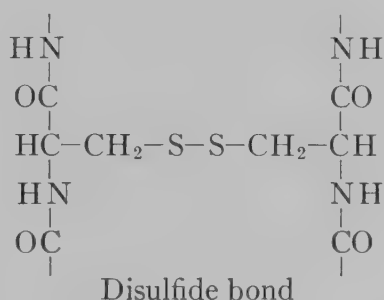


FIG. 7.—Disulfide cross-linkages

linkages, and between adjacent side chains and eventually the formation of new (unnatural) cross-linkages. If a fiber is kept stretched in steam for 2 minutes, it will remain elongated when removed from the steam, because of the destruction of contracting side-chain forces. If the fiber is kept for a short while in steam, unstretched, it will contract to about one-third less than its original length ("supercontraction"), again indicating a loosening of the structure but this time leading to a more extensive folding than that of the original α -keratin state (8).

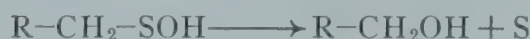
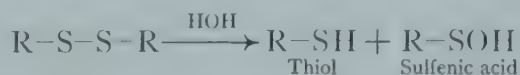
This supercontraction represents a modification of the molecular structure which is still reversible ("temporary set"). If, however, steaming or chemical action is prolonged or intensified, permanent new cross-linkages are built up, and the "free keratin" chains (residues of broken cross-bridges) are fixed again ("permanent set"). These phe-

nomena have been widely exploited in the so-called "cold-waving process" (19, 33).

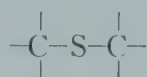
B. DISULFIDE CROSS-LINKAGES

The strongest linkages are the disulfide bonds connecting polypeptide chains cross-wise (Fig. 7). The disulfide bonds are mainly responsible for the resistance of keratins to

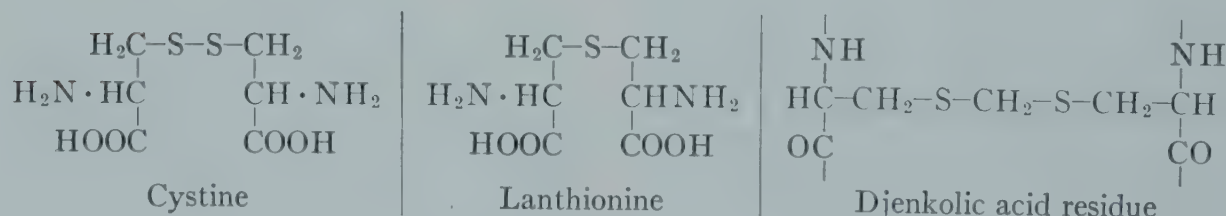
enzymatic digestion and other types of chemical disruption. When they are broken, a great variety of side-chain fragments are left, according to the nature and intensity of the destructive agents. Stoves (122) lists the following products of $-S-S-$ breakdown which were proved to occur by previous work of Schöberl *et al.* (110) and others:



If the fragments reunite to form a permanent set, as is the case in the cold-waving process, the most common newly formed unnatural cross-linkage type is the lanthionine linkage (71, 117):



Lanthionine is a compound closely resembling cystine but having only one atom of sulfur instead of two. After treatment with acids (120) or after treatment with reducing agents (92), the djenkolic acid type of cross-linkage can be obtained.



The re-formed keratins differ in their solubility, digestibility, and isoelectric point from the original keratin (61).

Medically, the breakage of disulfide bonds occurs when sulfides and thioglycolates are used as depilatories (95, 61) and when the latter are used in cold-waving (19, 33). All reducing agents act much more intensely at an alkaline pH. Speakman (116) demonstrated that the role of alkalinity is that of breaking the salt linkages (see below). The rupture of the salt linkages facili-

tates the penetration of the reducing agent to the otherwise less accessible disulfide bonds.

Concerning pathological events, it was stated that brittleness of the nails, when elicited by alkaline cleansers, may be associated with a breakage of disulfide linkages (38). Studies on the exposed tips of hair suggests, interestingly, that not only alkalies and other chemicals but also light and air may cause breakdown of disulfide bonds. According to Speakman (114), this breakdown probably occurs as follows:



The question arises as to whether a similar decomposition could also occur by light and air in the horny layer of the epidermis in man, particularly if the skin is not protected by the natural lipid film, as is the case in "asteatosis."

Not all disulfide bonds in a keratin molecule are equally accessible, and a variable percentage of the disulfide bonds is broken by different procedures. Schöberl *et al.* (110) maintained that, the methionine content of wool being negligible, almost all the sulfur in wool can be accounted for as cystine (or disulfide-bond) sulfur. Taking 100 per cent cystine sulfur as the reference point, the quotient of noncystine sulfur to total sulfur will represent the number of hydrolyzed di-

built into the chain; and free basic groups exist wherever basic diamino acids are built in. If a free acid and free basic group on neighboring polypeptide chains face each other in close proximity, there is an electrostatic attraction between the NH_3^+ group of the diamino acids, and the COO^- group of the dicarboxylic acids.

The example given in Figure 8 shows the simplest case of a salt linkage in which the free NH_3^+ group of a built-in lysine molecule attracts the free COO^- group of a built-in glutamic acid molecule. Speakman studied the salt linkages in great detail (116, 114).

From the biological point of view it is important to know that acids and alkalies at-

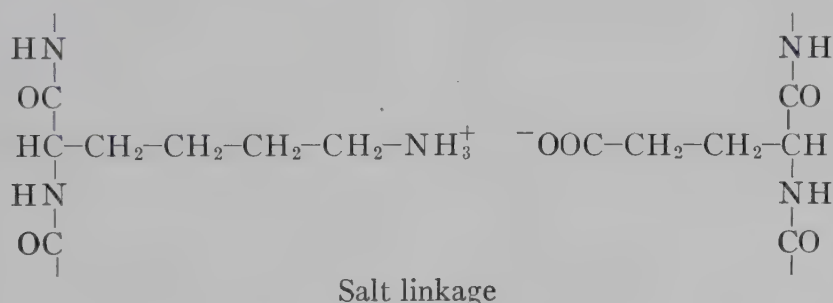


FIG. 8.—Salt linkage between glutamic acid and lysine residues

sulfide groups and will be a quantitative index of the damage to the fiber. Phillips (93) succeeded in dividing the bound cystine of wool into four subfractions, differing in chemical reactivity. It appears that not all the disulfide bonds act as cross-links between the micelles but that some are present within the micelles.

Alexander *et al.* (7, 4) on a new principle, distinguished two widely different cystine groups in wool: one which is oxidized by acid permanganate and alkaline hypochlorite (25 per cent) and one which is not (75 per cent). They find that the reactivity of the disulfide groups is dependent on induced electrical effects from neighboring groups or, in other words, on their "molecular environment."

C. SALT CROSS-LINKAGES

Free carboxylic groups occur in polypeptide chains whenever a dicarboxylic acid is

tack primarily these salt linkages. The swelling of keratin fibers in acid and alkaline solutions is explained by primary rupture of these bonds, whereby water can freely enter the intermicellar spaces.

D. HYDROGEN BONDS

The hydrogen bond or "hydrogen bridge" (130) is an interaction between two dipoles, one of which has a hydrogen atom at its positive end, while the other has a very strongly electronegative end, with an oxygen atom or, in some cases, a nitrogen atom. The attraction between two such dipoles is much greater than between dipoles in general, because the small size of the hydrogen atom permits it to approach the negative end of the other dipole much more closely than can any other electropositive atom. Figures 9 and 10 illustrate the positions of hydrogen bonds in polypeptide chains and in cross-linkages, respectively. The three main types

of cross-bonds in keratin are shown in one scheme in Figure 11.

In the last ten years more and more attention has been paid to alterations in the physical properties of wool fibers, to changes in solubility, swelling, elasticity, supercontraction, and permanent-set behavior which could not be explained by breakage of disul-

strength. True elasticity is obtained only after the breaking of hydrogen bonds. The theory postulates that the micelles of wool fibers are cylindrical, with the main polypeptide chains in the periphery (in the β -pattern) running parallel to one another and held together laterally by hydrogen bonds. This cylinder is the most unreactive part of

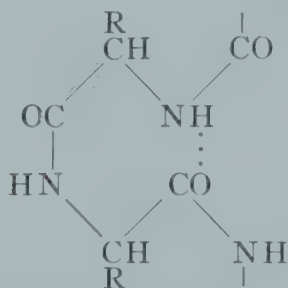


FIG. 9.—Hydrogen bond within the polypeptide chain.

fide bonds or salt linkages (9, 51, 66, 3, 4, 5) and which were attributed to reversible and irreversible breakages of hydrogen bonds. This gradually led to a new conception of the structure of the keratin molecule, culminating in the new theory of Alexander (4). According to this theory, the α -folding of keratins, as already indicated by Astbury (9), is due to hydrogen bonds, and the dry strength of the fiber depends almost wholly on hydrogen bonds and is hardly affected by energetic oxidative rupture of the disulfide linkages. Both disulfide and hydrogen bonds, however, contribute to the wet

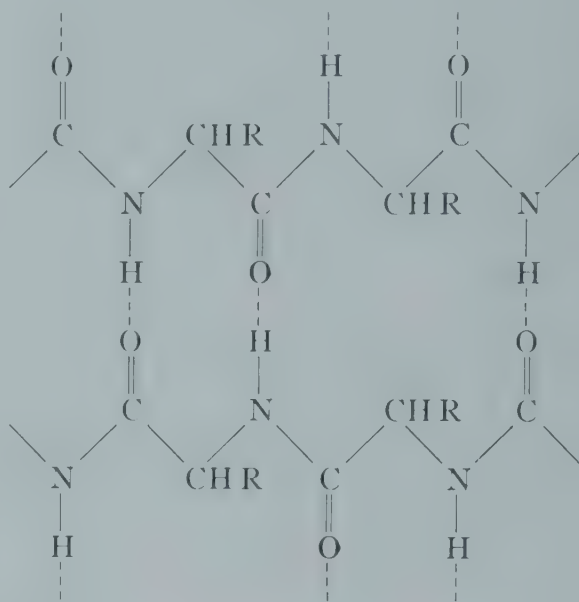


FIG. 10.—Hydrogen bonds in cross-linkages

the fiber and corresponds to the cuticle of the hair. Nonpolar side chains point into the center of the cylinder, and polar side chains point outward, as in soap micelles.

Alexander's theory has also touched upon

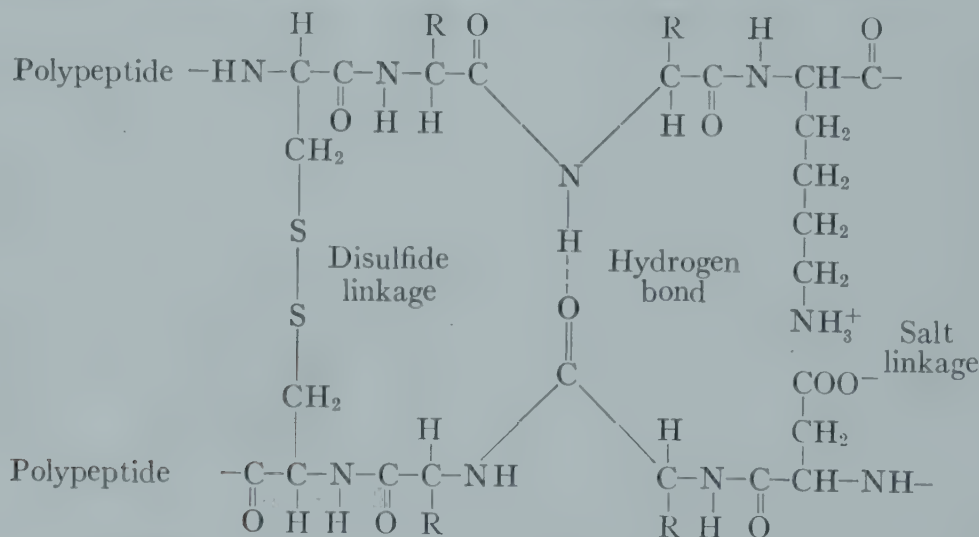


FIG. 11 (33).—The three main types of cross-linkages in keratin molecules

the biological problem of keratin formation by his assumption that the noncornified protein molecules, containing sulfhydryl groups, aggregate in solution into the cylindrical micelle, becoming cross-linked to the micelle by the formation of disulfide bonds. Thereby the noncornified material loses its original gel-like character and becomes a wool fiber. The theory, however, does not deal with the origin of the cylindrical membrane (or cuticle), which obviously also stems from living protoplasm.

A highly practical implication of this new concept has been the discovery that, by

treatment with strong lithium bromide solutions, keratin molecules are reversibly loosened by breakage of hydrogen bonds (3, 5, 4). For dermatologists this finding indicated that such solutions may be used as a new class of keratolytic agents in dermatotherapy. It was demonstrated in my laboratory (104) that, by treatment with strong lithium bromide solutions, human hair can be made sufficiently permeable to permit the killing of fungi inside the hair. The keratolytic effect of lithium bromide has also been utilized in the treatment of fungus infections of nails (103).

VI. ENZYMATIC DIGESTION OF KERATINS

In spite of the great resistance of keratins to enzymatic hydrolysis, there are some remarkably exceptional proteolytic enzymes which do perform this task. Linderstrom-Lang *et al.* (77, 78, 44) demonstrated a powerful proteinase in the middle intestine of common clothes-moth larvae (*Tineola biselliella*), which digests hard keratins. The optimum pH at 40° C. is 9.3. The enzyme, as contrasted with pancreatic tryptic enzymes, is not inhibited by sulphydryl groups. Thus, if there is a primary breakdown of disulfide linkages to sulphydryl groups (p. 356), further enzymatic decomposition may be attained. The explanation for the activity of this enzyme in vivo is that the intestine contains a strong reducing agent. This was shown by feeding oxidoreductive indicators to living larvae. It is probably this reducing agent which breaks the disulfide bonds and renders the proteins accessible to the enzymatic action of the intestinal keratinase of the moth. This is quite a special situation in the animal world. Otherwise, in vertebrates as well as in invertebrates, keratins cannot be utilized because the gastrointestinal juices are unable to hydrolyze them.

Fungi are more powerful in this respect. Jensen (73) demonstrated the decomposition of keratins by two *Actinomyces* strains—*A. citreus* Krainsky and one strain closely related to Waksman's *Actinomyces* 145, both occurring in soil. These organisms thrive on

pure keratin, forming ammonia and, in the presence of a mixed flora, also forming nitrates. Even more important is a recent finding by Stahl *et al.* (118), demonstrating the presence of a keratinase in the fungus *Microsporum gypseum*, which is pathogenic for human skin.⁴ In decreasing order of efficacy, it digests collagen, feathers, hoof, wool, horn, horsehair, and silk. The enzyme has endo- and exoproteinase components, all different from trypsin.

It should be noted that the hair of various mammals, including man, is not perfectly resistant to tryptic digestion. Elöd and Zahn (52) found that 10 per cent of the nitrogenous material of sheep wool is solubilized by pancreatin; and Stoves (121) pointed out that if hair is cross-sectioned, the medulla can be completely disintegrated by trypsin. Because cuticle and cortex withstand this action on the outside and because trypsin has no access to the medulla, the whole hair appears to be indigestible. Castellino (35, 36) experimented with the proteinase which can be extracted from mammalian epidermis (p. 704) but found that it does not digest keratin.

4. See also R. M. Page, Observations on keratin digestion by *Microsporum gypseum*, *Mycologia*, **42**: 591–602, 1950; L. Ajello, The dermatophyte, *Microsporum gypseum*, as a saprophyte and parasite, *J. Invest. Dermat.*, **21**:157–71, 1953.

VII. CLASSIFICATION OF KERATINS

The first attempt to classify keratins was that of Unna (124, 125). His classification into keratin A, keratin B, keratin C, and keratin-albumose was based on different resistance to hydrolyzing and oxidizing agents. Keratin A withstands even treatment with a mixture of 40 per cent sulfuric acid and 10 per cent hydrogen peroxide for 24 hours and is found in the outermost layers of hoofs, claws, horns, nails, and in hair cuticles and feather cuticles. Keratin B, in contrast to A,

TABLE 8

TWO TYPES OF KERATIN (58)

Hard	Soft
Not desquamating	Desquamating
High sulfur content	Low sulfur content
High cystine content	Low cystine content
More sensitive to Na_2S	Less sensitive to Na_2S
Does not reduce KMnO_4	Reduces KMnO_4
Fat content low	Fat content high

is decomposed by strong acids and weak alkalies and occurs on the inside of horns, hoofs, and claws. Keratin C is attacked by alkalies, but not by concentrated nitric acid, and is present inside hairs and feathers. Keratin-albumoses are the not fully keratinized parts of horny cells. They are easily soluble in nitric acid or in a sulfuric acid-hydrogen peroxide mixture and can be reprecipitated by phosphotungstic acid or by saturation with sodium chloride. In the horny layer of human plantar skin, Unna found 13 per cent keratin A, 10 per cent keratin B, and abundant amounts of albumose.

A second classification scheme was that of Giroud, Bulliard, and Leblond (58). They attempted to establish consistent differences between soft and hard keratin structures, not denying, however, that transitional features make such a classification difficult. Biologically, their main points were that soft keratins, such as those of the horny layer, do desquamate, while hard keratins, belonging to the appendages, do not, and that hard keratins are formed rather simply (without interposition of a granular layer), while the formation of soft keratins is complicated, the process passing through a number of consecutive phases. They suggested that the process producing keratins which disaggregate spontaneously (i.e., desquamate) should be called "schizokeratinization," as contrasted with "sclerokeratinization," in which nondesquamating horny material is produced. The chemical and histochemical differences found by Giroud *et al.* are tabulated in Table 8.

In view of modern knowledge, it is obvious that keratinization is a continuous progressive process and that it is a matter of the degree of keratinization which determines how the end-products will behave. Therefore, it is rather hopeless to establish categories. Even the distinction of desquamating and nondesquamating keratins is a relative one. Callosities, for instance, are pathological variants of soft epidermal keratins and certainly desquamate less than normal keratin of the horny layer (p. 378).

BIBLIOGRAPHY

1. ABDERHALDEN, E., and HEYNS, U. Nachweis von Methionine im Keratin des Rinderhornes, *Ztschr. f. physiol. Chem.*, **207**:191-92, 1932.
2. ADOLPH, W. E.; BAKER, R. F.; and LEIBY, G. M. Electron microscope study of epidermal fibers, *Science*, **113**:685-86, 1951.
3. ALEXANDER, P. Role of hydrogen bonds in the supercontraction and permanent set of wool fibers, *Research*, **2**:246-47, 1949.
4. ———. Changes in the physical properties of wool fibers produced by breaking hydrogen bonds with lithium bromide solutions, *Ann. New York Acad. Sc.*, **53**:653-73, 1951.
5. ———. Beziehungen zwischen Struktur und chemischer Reaktionsfähigkeit von Wollfasern, *Kolloid Ztschr.*, **122**:8-19, 1951.
6. ALEXANDER, P., and EARLAND, C. Structure of wool fibers. Isolation of an alpha

- and beta protein in wool, *Nature*, **166**: 396-97, 1950.
7. ALEXANDER, P.; HUDSON, R. F.; and FOX, M. The reaction of oxidizing agents with wool. 1. The division of cystine into two fractions of widely differing reactivities, *Biochem. J.*, **46**:27-32, 1950.
 8. ASTBURY, W. T. Fundamentals of fibre structure. London: Oxford University Press, Humphrey Milford, 1933.
 9. ———. The hydrogen bond in protein structure, *Tr. Faraday Soc.*, **36**:871-80, 1940.
 10. ———. X-rays and the stoichiometry of the proteins, with special reference to the structure of the keratin-myosin group, *J. Chem. Soc.*, pp. 337-47, 1942.
 11. ———. Essays on growth and form. London: Oxford University Press, 1945.
 12. ———. The molecular structure and elastic properties of hair. *In*: SAVILL, A., *The hair and the scalp*, pp. 62-74. Baltimore: Williams & Wilkins Co., 1945.
 13. BAILEY, K.; ASTBURY, W. T.; and RUDALL, K. M. Fibrogen and fibrin as members of the keratin-myosin group, *Nature*, **151**:716-17, 1943.
 14. BALDWIN, E. Dynamic aspects of biochemistry. London: Cambridge University Press, 1949.
 15. BARRITT, J. Distribution and origin of sulfur in wool. I. Methionine in wool, *Biochem. J.*, **28**:1-5, 1934.
 16. BERGMANN, M. Proteins and proteolytic enzymes, *Harvey Lect.*, **31**:37-56, 1931.
 17. BERGMANN, M., and NIEMANN, C. On the structure of proteins: cattle hemoglobin, egg albumin, cattle fibrin gelatin, *J. Biol. Chem.*, **118**:301-14, 1937.
 18. ———. On the structure of silk fibroin, *ibid.*, **122**:577-96, 1938.
 19. BERGY, G. A. The action of thioglycolates, *Am. Prof. Pharmacist*, **14**:1014-18, 1056-57, 1948.
 20. BEVERIDGE, J. M. R., and LUCAS, C. C. The analysis of hair keratin. II. The dicarboxylic and basic amino acids of human hair, *Biochem. J.*, **38**:88-95, 1944.
 21. ———. Analysis of hair keratin. III. Isolation of proline from human hair, *ibid.*, pp. 95-97.
 22. BLOCK, R. J. Basic amino acids of human skin, *Proc. Soc. Exper. Biol. & Med.*, **32**: 1574-75, 1935.
 23. ———. The nature and origin of protein, *Yale J. Biol. & Med.*, **7**:235-52, 1935.
 24. ———. Chemical studies on neuroproteins; on nature of proteins of ektoderm; eukeratins and pseudokeratins, *J. Biol. Chem.*, **121**:761-70, 1937.
 25. ———. The composition of keratins; the amino acid composition of hair, wool, horn and other eukeratins, *ibid.*, **128**:181-86, 1939.
 26. ———. The chemical constitution of the proteins. *In*: SCHMIDT, C. L. A. *The chemistry of the amino acids and proteins*, pp. 278-333. 2d ed. Springfield, Ill.: Charles C Thomas, 1944.
 27. BLOCK, R. J., and BOLLING, D. The amino acid composition of proteins and foods. Analytical methods and results. 2d ed. Springfield, Ill.: Charles C Thomas, 1951.
 28. BLOCK, R. J., and VICKERY, H. B. The basic amino acids of proteins. A chemical relationship between the various keratins, *J. Biol. Chem.*, **93**:113-17, 1931.
 29. BOLLIGER, A. Water-extractable constituents of hair, *J. Invest. Dermat.*, **17**:79-84, 1951.
 30. BONTING, S. J. L., JR. Cysteine, cystine and methionine in the skin of young and adult rats, *Biochim. et biophys. acta*, **6**: 183-86, 1950.
 31. BORCHI, B. Die Haut als Stoffwechselorgan der Aminosäuren (die oxydative Spaltung des Arginins), *Klin. Wchnschr.*, **18**: 507-8, 1939.
 32. ———. Ulteriori ricerche sul metabolismo degli amino acidi nella cute. "Il triptofano," *Riv. di biol.*, **30**:262-64, 1940.
 33. BRUNNER, M. J. Medical aspects of home cold waving, *Arch. Dermat. & Syph.*, **65**: 316-26, 1952.
 34. CARRIÉ, A. B. D. Water absorption of keratin. *In*: *Fibrous proteins: proceedings of a symposium held at the University of Leeds, May, 1946*, pp. 86-95. Bradford: Society of Dyers and Colourists, 1946.
 35. CASTELLINO, P. G. The action of enzymic extracts on the solubility of keratin, *Arch. Ist. biochim. ital.*, **7**:417-20, 1935. Quoted in *Chem. Abstr.*, **30**:3839, 1936.
 36. ———. The effect of autolyzed skin extracts on dissolved keratin. II. Papain enzyme, *ibid.*, **9**:171-74, 1937. Quoted in *Chem. Abstr.*, **31**:7893, 1937.
 37. CHAMBERS, R., and RENYI, S. G. Structure of the cells in tissue as revealed by

- microdissection, *Am. J. Anat.*, **35**:385-402, 1925.
38. CHIEGO, B., and SILVER, H. Effect of alkalies on the stability of keratins, *J. Invest. Dermat.*, **5**:95-103, 1942.
 39. COHEN, M. H. A clinical appraisal of the cold wave process, *Arch. Dermat. & Syph.*, **60**:14-23, 1949.
 40. COHN, E. J., and EDSALL, J. T. *Proteins, amino acids and peptides as ions and dipolar ions*. New York: Reinhold Publishing Corp., 1943.
 41. CONANT, J. B., and BLATT, A. H. *The chemistry of organic compounds*. 3d ed. New York: Macmillan Co., 1948.
 42. DERKSEN, J. C., and HERINGA, G. C. Cornification and tonofibrils of epidermis, *Polska gaz. lek.*, **15**:592-94, 1936.
 43. DERKSEN, J. C.; HERINGA, G. C.; and WEIDINGER, A. On keratin and cornification, *Acta néerl. morph. norm. et path.*, **1**:31-37, 1937.
 44. DUSPIVA, F. Beiträge zur enzymatischen Histochemie. XXI. Die proteolytischen Enzyme der Kleider- und Wachsmottenraupen, *Ztschr. f. physiol. Chem.*, **241**:177-200, 1936.
 45. ECKSTEIN, H. C. Amino acids in human skin, *Proc. Soc. Exper. Biol. & Med.*, **32**:1573-74, 1935.
 46. ECKSTROM, P. E., JR. Swelling studies of single human hair fibers, *J. Soc. Cosmet. Chemists*, **2**:244-49, 1951.
 47. EICHELBERGER, L., and ROMA, M. Electrolyte and nitrogen distribution in whole fat free skin and heat-separated corium and epidermis, *J. Invest. Dermat.*, **12**:125-38, 1949.
 48. EISELE, C. W., and EICHELBERGER, L. Water, electrolyte and nitrogen content of human skin, *Proc. Soc. Exper. Biol. & Med.*, **58**:97-100, 1945.
 49. ELÖD, E.; NOWOTNY, H.; and ZAHN, H. Structure and reactivity of the woolen fiber. IX. The effect of H₂O₂ on wool, *Melliand Textilber.*, **23**:313-16, 1942. Quoted in *Chem. Abstr.*, **38**:3136, 1944.
 50. ELÖD, E., and ZAHN, H. Structure and reactivity of wool keratin. XIV. The preparation and properties of wool spindle cells, *Melliand Textilber.*, **24**:245-49, 1943. Quoted in *Chem. Abstr.*, **38**:2825, 1944.
 51. ———. Structure and reactivity of wool fiber. XVI. Action of formamide on keratins: "Supercontraction," *Kolloid Ztschr.*, **108**:94-103, 1944. Quoted in *Chem. Abstr.*, **40**:6903, 1946.
 52. ———. The action of pancreatin on fiber keratin, *Melliand Textilber.*, **25**:361-63, 1944. Quoted in *Chem. Abstr.*, **40**:5080, 1946.
 53. FISCHER, E. *Untersuchungen über Aminosäuren, Polypeptide und Proteine*. Berlin: J. Springer, 1906.
 54. FRIEBOES, W. Beiträge zur Anatomie und Biologie der Haut. X. Die biologischen Funktionen der menschlichen Epidermis, *Arch. f. Dermat. u. Syph.*, **140**:467-80, 1922.
 55. GEIGER, W. B. The scale substance of wool, *Textile Research*, **14**:82-85, 1944.
 56. GILBERT, G. A. A criticism of some theories of multi-molecular adsorption applied to wool. In: *Fibrous proteins: proceedings of a symposium held at the University of Leeds, May, 1946*, pp. 86-95. Bradford: Society of Dyers and Colourists.
 57. GIROUD, A., and BULLIARD, H. La kératinisation de l'épiderme et des phanères. Paris: G. Doin, 1930.
 58. GIROUD, A.; BULLIARD, H.; and LEBLOND, C. P. Les deux types fondamentaux de kératinisation, *Bull. d'histol. appliq. à la physiol.*, **11**:129-44, 1934.
 59. GIROUD, A., and CHAMPETIER, G. Recherches sur les roentgenogrammes des kératines, *Bull. Soc. chim. biol.*, **18**:655-64, 1936.
 60. GIROUD, A., and LEBLOND, C. P. The keratinization of epidermis and its derivatives, especially the hair, as shown by X-ray diffraction and histochemical studies, *Ann. New York Acad. Sc.*, **53**:613-26, 1951.
 61. GODDARD, D. R., and MICHAELIS, L. A study on keratin, *J. Biol. Chem.*, **106**:605-14, 1934.
 62. GRAY, M.; BLANK, H.; and RAKE, G. Electron microscopy in normal human skin, *J. Invest. Dermat.*, **19**:449-57, 1952.
 63. GREENSTEIN, J. P. *Biochemistry of cancer*. New York: Academic Press, 1947.
 64. GRZYCKI, S. The tannophilic fibers of the human skin and their relation to the epidermis cells, *Ann. Univ. Mariae Curie-Skłodowska, Sec. D—Med.*, **4**:53-67, 1949.
 65. HARRIS, M. Chemistry of keratin, *J. Soc. Cosmet. Chemists*, **1**:225-30, 1949.
 66. HARRIS, M., and BROWN, A. E. Natural

- and synthetic protein fibers. *In*: Fibrous proteins: proceedings of a symposium held at the University of Leeds, May, 1946. Bradford: Society of Dyers and Colourists, 1946.
67. HARRIS, M.; MIZELL, L. R.; and FOURT, L. Elasticity of wool as related to its chemical structure, *J. Res. Nat. Bur. Standards*, **29**:73-86, 1942.
 68. HAUROWITZ, F. Chemistry and biology of proteins. New York: Academic Press, 1950.
 69. HESS, W. C. Variations in amino-acid content of fingernails of normal and arthritic individuals, *J. Biol. Chem.*, **109**:xliii, 1935.
 70. HOFMEISTER, F. Über Bau und Gruppierung der Eiweisskörper, *Ergebn. d. Physiol.*, **1**:759-802, 1902.
 71. HORN, M. J.; JONES, D. B.; and RINGEL, S. J. Isolation of a new sulfur-containing amino acid (lanthionine) from sodium carbonate-treated wool, *J. Biol. Chem.*, **138**:141-49, 1941.
 72. JACOBS, J. L. A modified technique for determining protein fractions of animal skin, *J. Am. Leather Chemists A.*, **44**:722-37, 1949.
 73. JENSEN, H. L. Decomposition of keratin by soil micro-organisms, *J. Agr. Sc.*, **20**:390-98, 1930.
 74. KAWABE, M. Studien über die Verhornung des menschlichen Haut. II. Mikrochemische Untersuchungen der Hornschicht bei normaler und scheinbar normaler Haut mit Hilfe des Millons Reagens, *Jap. J. Dermat. & Urol.*, **36**:117-18, 1939.
 75. KOPSCH, FR. Anatomie des Menschen, Vol. 6. 9th ed. Leipzig: G. Thieme, 1912.
 76. LADEN, E. L. Electron microscopic study of epidermal prickle cells, *J. Invest. Dermat.*, **19**:211-15, 1952.
 77. LINDERSTROM-LANG, K., and DUSPIVA, F. Keratin digestion in the larvae of the clothes moth, *Nature*, **135**:1039-40, 1935.
 78. ———. Beiträge zur enzymatischen Histochemie. XVI. Die Verdauung von Keratin durch die Larven der Kleidermotte (*Tineola biselliella* Humm), *Ztschr. f. physiol. Chem.*, **237**:131-58, 1935.
 79. LLOYD, D. J., and GARROD, M. Structure of protein fibers with special reference to the so-called thermal shrinkage of the collagen fiber, *Tr. Faraday Soc.*, **44**:441-50, 1948.
 80. LUCAS, C. C., and BEVERIDGE, J. M. R. The analysis of hair keratin. I. A method for the quantitative removal of cystine from keratin hydrolysates, *Biochem. J.*, **34**:1356-66, 1940.
 81. MANDER, J. V., and BROWN, H. Certain phases of sulfur metabolism of the skin, *Arch. Dermat. & Syph.*, **34**:568-81, 1936.
 82. MANGOLD, E., and DUBISKI, J. Die Verdauung des Keratins, besonders der Hornsubstanz von Vogelfedern, durch Säugetiere und Vogel, *Wissensch. Arch. Landw., Abt. B, Tierernähr. u. Tierzucht*, **4**:200-221, 1930.
 83. MARDASHEV, S. R. Amino acid composition of the proteins of the skin, epidermis and dermis, *Biokhimiya*, **12**:444-51, 1947. Quoted in *Biol. Abstr.*, **24**:18768, 1950.
 84. MARDASHEV, S. R., and SEMINA, L. A. Arginase in the skin, *Biokhimiya*, **13**:236-43, 1948. Quoted in *Chem. Abstr.*, **42**:7806, 1948.
 85. MARSTON, H. R. Nutrition and wool production. *In*: Fibrous proteins: proceedings of a symposium held at the University of Leeds, May, 1946, pp. 207-14. Bradford: Society of Dyers and Colourists, 1946.
 86. MARTIN, A. J. P. A new approach to the problem of the structure of proteins. An investigation of a partial hydrolysate of wool. *In*: Fibrous proteins: proceedings of a symposium held at the University of Leeds, May, 1946, pp. 1-14. Bradford: Society of Dyers and Colourists, 1946.
 87. MERCER, E. H., and REES, A. L. G. Structure of the cuticle of wool, *Nature*, **157**:589-90, 1946.
 88. ———. An electron microscope investigation of the cuticle of wool, *Australian J. Exper. Biol. & Med. Sc.*, **24**:147-58, 1946.
 89. MESCON, H., and FLESCH, P. Modification of Bennett's method for the histochemical demonstration of free sulfhydryl groups in skin, *J. Invest. Dermat.*, **18**:261-66, 1952.
 90. NADEL, A. Chemische Studien an der menschlichen Haut. II. Untersuchungen an der normalen und der pathologischen Menschenhaut, *Arch. f. Dermat. u. Syph.*, **165**:507-24, 1932.
 91. NEUMAN, R. A., and LOGAN, M. A. The determination of collagen and elastin in tissues, *J. Biol. Chem.*, **186**:549-56, 1950.
 92. PATTERSON, W. I.; GEIGER, W. B.; MIZELL, L. R.; and HARRIS, M. Role of cystine in the structure of the fibrous protein, wool,

- J. Res. Nat. Bur. Standards, **27**:89-103, 1941.
93. PHILLIP, H. The division of the combined cystine of wool into four subfractions differing in chemical reactivity. *In*: Fibrous proteins: proceedings of a symposium held at the University of Leeds, May, 1946, pp. 39-49. Bradford: Society of Dyers and Colourists, 1946.
 94. PINKUS, F. Die normale Anatomie der Haut. *In*: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1/1**:1-378. Berlin: J. Springer, 1927.
 95. PULEWKA, P. Weitere Untersuchungen über Keratolyse, Arch. f. exper. Path. u. Pharmakol., **140**:181-93, 1929.
 96. RALPH, P. H. Observations on the surface of epithelial cells, Anat. Rec., **98**:219-21, 1947.
 97. ROBERTS, E.; CALDWELL, A. L.; CLOWES, G. H. A.; SUNTZEFF, V.; CARRUTHERS, C.; and COWDRY, E. V. Amino acids in epidermal carcinogenesis in mice, Cancer Research, **9**:350-53, 1949.
 98. ROBERTS, E., and FRANKEL, S. Urea and ammonia content of mouse epidermis, Arch. Biochem., **20**:386-93, 1949.
 99. ———. Arginase activity and nitrogen content in epidermal carcinogenesis in mice, Cancer Research, **9**:231-37, 1949.
 100. ROBERTS, E., and TISHKOTT, G. H. Distribution of free amino acids in mouse epidermis in various phases of growth as determined by paper partition chromatography, Science, **109**:14-16, 1949.
 101. RODDY, W. T. The coagulable proteins of animal skin, J. Am. Leather Chemists A., **37**:410-26, 1942.
 102. ROTHMAN, S. Discussion on S. A. M. JOHNSON and N. Y. GRIMM, The amino acid requirements of *Microsporum fulvum*, J. Invest. Dermat., **17**:305-10, 1951.
 103. ———. Studies on systemic disturbances and therapeutic experiments on recalcitrant *Trichophyton purpureum* infections; with a short report on therapeutic experiments, Arch. Dermat. & Syph., **67**:239-46 and 259-62, 1953.
 104. ROTHMAN, S., and TASCHDJIAN, C. L. A new method to increase the permeability of hard keratin structures, J. Invest. Dermat., **17**:9-11, 1951.
 105. RUDALL, K. M. The structure of epidermal proteins. *In*: Fibrous proteins: proceedings of a symposium held at the University of Leeds, May, 1946, pp. 15-23. Bradford: Society of Dyers and Colourists, 1946.
 106. ———. X-ray studies of the distribution of protein-chain types in the vertebrate epidermis, Biochim. et biophys. acta, **1**:549-62, 1947.
 107. ———. The proteins of the mammalian epidermis, Adv. in Protein Chem., **7**:253-59, 1952. New York: Academic Press, Inc.
 108. SCHMIDT, C. L. A. The chemistry of the amino acids and proteins. 2d ed. Springfield, Ill.: Charles C Thomas, 1944.
 109. SCHMIDT, W. J. Neue Polarisationsoptische Arbeiten auf dem Gebiete der Biologie, Protoplasma, **29**:300-320, 1937.
 110. SCHÖBERL, A., and RAMBACHER, P. Über die Reaktionsfähigkeit des Keratins der Schafwolle, Biochem. Ztschr., **306**:269-95, 1940.
 111. SPEAKMAN, J. B. Micelle structure of the wool fiber, Proc. Roy. Soc. London, s.A., **132**:167-91, 1931.
 112. ———. Reactivity of the sulfur linkage in wool, Nature, **132**:930, 1933.
 113. ———. Cross-linkage formation in keratins, *ibid.*, **138**:327, 1936.
 114. ———. Mechano-chemical methods for use with animal fibers, J. Textile Inst., **38**:T102-T126, 1947.
 115. SPEAKMAN, J. B., and ELLIOTT, G. H. The combination of wool with acids and acid dyes. *In*: Fibrous proteins: proceedings of a symposium held at the University of Leeds, May, 1946, pp. 116-28. Bradford: Soc. of Dyers and Colourists, 1946.
 116. SPEAKMAN, J. B., and TOWNSEND, F. Constitution of the keratin molecule, Nature, **139**:411-12, 1937, and **141**:414, 1938.
 117. SPEAKMAN, J. B., and WHEVELL, C. S. The reactivity of the sulfur linkage in animal fibers. II. The action of baryta and caustic soda on human hair, J. Soc. Dyers & Colourists, **52**:380-88, 1936.
 118. STAHL, W. H.; MCQUE, B.; MANDELS, G. R.; and SIU, G. H. Studies on the microbiological degradation of wool digestion of normal and modified fibrillary proteins, Textile Res. J., **20**:570-79, 1950.
 119. STOVES, J. L. Structure of keratin fibers, Nature, **151**:304-5, 1943.
 120. ———. The reactivity of the cystine linkage in keratin fibers. IV. The action of formaldehyde, Tr. Faraday Soc., **39**:294-300, 1943.
 121. ———. The chemistry of animal hair. *In*:

- Fibrous proteins: proceedings of a symposium held at the University of Leeds, May, 1946, pp. 58-66. Bradford: Society of Dyers and Colourists, 1946.
122. ———. Some biological and chemical properties of animal hair, *J. Soc. Dyers & Colourists*, **63**:65-77, 1947.
123. TARANTINO, C. Il ricambio della arginina nella cute degli uccelli, *Riv. di biol.*, **30**: 290-93, 1940.
124. UNNA, P. G. Die Bedeutung der Hornschicht, *Med. Klin.*, **16**:1276-77, 1920.
125. ———. Die hornerweichenden und hornlösenden Mittel, *Deutsche med. Wchnschr.*, **47**:822-24, 1921.
126. UNNA, P. G., and SCHUMACHER, J. Lebensvorgänge in der Haut des Menschen und der Tiere. Leipzig and Vienna: F. Deuticke, 1925.
127. URBACH, E. Beiträge zu einer physiologischen und pathologischen Chemie der Haut, *Arch. f. Dermat. u. Syph.*, **156**:73-101, 1928.
128. VAN SCOTT, E. J. Studies on the arginase activity of the skin, *J. Invest. Dermat.*, **17**:21-26, 1951.
129. WEIDINGER, A. Investigations on the structure of proteins, *Collegium*, **837**:1, 1940.
130. WHELAND, G. W. Advanced organic chemistry. 2d ed. New York: John Wiley & Sons, 1949.
131. WILKERSON, V. A. Chemistry of human epidermis; amino acid content of stratum corneum and its comparison to other human keratins, *J. Biol. Chem.*, **107**:377-81, 1934.
132. WILKERSON, V. A., and TULANE, V. J. The chemistry of human skin. III. The occurrence of methionine in human skin (stratum corneum), *J. Biol. Chem.*, **129**:477-79, 1939.

CHAPTER 16

The Keratinization Process

I. TRANSFORMATION OF CYTOPLASMIC PROTEINS INTO KERATINS	367
A. Tonofibrils and the Keratinization Process	367
B. The Formation of Disulfide Cross-Linkages	368
C. The Disappearance of Sulfhydryl Groups and the Kera- togenous Zone of Giroud	369
D. The Catalytic Effect of Copper in the Formation of Disul- fide Cross-Linkages	371
E. The Problem of Absolute Increase in Sulfur Content and the Possibility of Hydrolytic Decomposition	372
F. Formation of Salt Linkages and Hydrogen Bonds	372
G. Glycogen Synthesis or Accumulation of Glycogen	373
H. Keratinization: A Denaturation Process	374
II. CELLULAR DECOMPOSITION DURING KERATINIZATION	374
A. Dehydration	374
B. Decomposition of Cytoplasmic Compounds	374
C. Decomposition of Nuclear Material	374
III. PARTICULATE ELEMENTS IN KERATINIZATION	375
IV. THE DESQUAMATION PROCESS AND ITS ANOMALIES	377
A. Normal Shedding	377
B. Formation of Visible Scales	377
C. Inhibition of Shedding	377
D. Chapping	378
V. THE EFFECT OF ESTROGENS	380
VI. THE EFFECT OF VITAMIN A	382
VII. KERATINIZATION IN VITRO	385

THE germinative cells of the epidermis, hairs, and nails have an intrinsic life-cycle. As they multiply and their daughter-cells approach the surface, they gradually change their character until their life terminates with the formation of dead anuclear horny cells. In the mammalian epidermis this dead end-product is continuously shed in the form of invisible tiny particles. The transformation of living epithelial cells into horny material is called the "keratinization" or "cornification" process. This process is one

of the most important facets of the biology of the skin.

In discussing the process of keratinization, it should be kept in mind that this process includes not only the transformation of cytoplasmic proteins into keratin fibers but also a complete disintegration of the keratinizing cell, including decomposition of both cytoplasm and nucleus. From the clinician's point of view, both features are equally important because either one or both can be disturbed in keratinization

anomalies. In faulty keratinization, disturbances of the cellular decomposition are probably at least as important as anomalies

of keratin formation. The two aspects will be discussed separately.

I. TRANSFORMATION OF CYTOPLASMIC PROTEINS INTO KERATINS

A. TONOFIBRILS AND THE KERATINIZATION PROCESS

Because most of the cellular proteins are globular in shape, or at least tightly coiled, one may be inclined to believe that keratinization involves straightening of coiled polypeptide chains; but it is not quite clear whether such straightening actually occurs, because possibly the tonofibrils are the forerunner elements of keratinization, which themselves are fibrous in structure. As mentioned (p. 344), tonofibrils show the same X-ray α -diagram as do keratin fibers, which indicates that they, too, consist of elongated chains. Histochemical observations suggest that tonofibrils are the crystallization centers of keratin formation (81, 82, 69).

Keratinization starts very early also in the periphery of the cell; as a matter of fact, the periphery undergoes the most intense keratinization, and it is not known whether there are preformed fibrous proteins in the cell "membrane." The difficulty in evaluating the histological observation is best illustrated by the remarkable findings of Szodora (79). He treated skin sections with tryptic enzymes and, after digestion, obtained a meshwork of lines corresponding with the contours of cells in the upper Malpighian layer. The cell content was completely washed out. These findings indicated that the cell periphery becomes increasingly trypsin-resistant as the rete cells ascend or, in other words, that it starts to keratinize in the upper Malpighian layer before the intracellular fibrils show a similar tendency. However, on close inspection, the cell contour lines proved to be composed of tiny nodules; and the possibility that the "lines" represented crossing points of epithelial fibrils (known in histology as the "Bizzozero nodules" or "Ranvier nodules") could not be excluded. Thus the concept that fibrils are

the primary site of keratinization was not ruled out.

Heringa *et al.* (22, 23) went so far as to assume that keratinization does not actually involve any protein conversion into keratin but rather an "unmixing and decomposition" of the fluid cell content, while the fibrils remain; the fibrils become coherent and thereby are converted into keratin. Giroud *et al.* (37) objected to this view on the basis of differences they had found in the sulfur content of nonkeratinized and keratinized epithelium. The view is objectionable, also, because the rete certainly does not contain elements which are as trypsin-resistant as keratin fibers are. The fact that tonofibrils have an α -keratin X-ray spectrum certainly does not mean that their chemical composition is identical with that of keratin. The α -picture is the same with and without increase in the number of disulfide bonds, and there are fibrous proteins with the α -keratin spectrum which never keratinize, such as myosin.¹

While it is possible that epidermal keratinization starts in the tonofibrils, there can be little doubt that these fibrils have to undergo further changes in order to become keratin fibers. That there are considerable differences in the physical behavior of epidermal fibrils and horny fibers has been conclusively demonstrated in the recent work of Rudall (p. 346).

The simultaneous initial keratinization in the cell periphery requires further elucidation.

1. Mercer found that in a relatively early phase of hair formation, in the so-called "unstabilized fibrous region," the same α -pattern is present as in the final hair. Total birefringence and X-ray pattern are not affected by the ensuing consolidation and hardening of the hair fiber (E. H. Mercer, Some experiments on the orientation and hardening of keratin in the hair follicle, *Biochim. et biophys. acta*, 3:161-69, 1949). For differences in the properties of "epidermin" and of keratin see also p. 346.

tion. Whether the keratinization of appendages starts from preformed fibrils or from globular proteins is not known. Marston (48) found no evidence whatsoever of preferred orientation in the contents of germinative cells prior to hair formation, which implies that keratinization does involve the straightening of coiled chains. Interestingly, however, he found that the cortical cells become anisotropic (meaning a preferred direction of orientation) before the keratin is set by oxidative closure of $-SH$ groups.

B. THE FORMATION OF DISULFIDE CROSS-LINKAGES

It was emphasized on page 351 that the only consistent chemical difference between

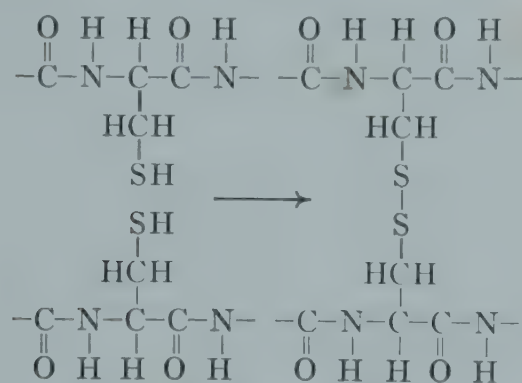


FIG. 1.—Formation of disulfide bond

the composition of keratins and that of cell proteins, from which keratin derives, is the higher cystine content of keratins. The increase in cystine content is associated with a decrease in sulfhydryl-containing amino acids. By far the largest part of the cystine-sulfur in the keratin molecule is present in the form of disulfide cross-linkages. Therefore, even though it never has been shown directly, there can be little doubt that during keratinization sulfhydryl-containing amino acid residues facing each other on neighboring polypeptide chains will close to form a disulfide cross-linkage. There is probably quite general agreement that this reaction is the paramount chemical feature of keratin formation. Cohn and Edsall (19) present the scheme shown in Figure 1 to illustrate the formation of disulfide bonds.

In this scheme the sulfhydryl-containing amino acid residues represent cysteine; and when their adjacent $-SH$ groups are oxidized to form an $-S-S-$ group, the cysteine residues will form a cystine residue which bridges over two polypeptide chains. This transformation is rather simple and easy to understand and accounts for the diminution of $-SH$ groups in keratinization. It is more difficult, however, to account for the lessening of the amount of methionine, as doubtless occurs in keratinization (p. 369).

Sulfur in methionine has a position in the molecule quite different from its position in cysteine, being situated between a CH_2 and a CH_3 group. In such a position, sulfur is not labile. For instance, it cannot be split off by alkali, as contrasted with cysteine-sulfur and cystine-sulfur.

It has been known for half a century from the work of the Moerners (56, 57) that proteins contain sulfur in two different forms:² A labile sulfur, which is liberated in hot alkaline solution as sulfide ion, and a nonlabile sulfur, which yields methylsulfonic acid when proteins are treated with nitric acid. The nature of the nonlabile sulfur remained unknown until 1922, when the new sulfur-containing amino acid, methionine, was isolated from protein hydrolysates by J. H. Mueller (62, 63).

From our point of view, the problem is whether methionine in protein can be utilized for the formation of disulfide cross-linkages, as cysteine is. Again, the actual occurrence of such a process has not been demonstrated, but it certainly could occur, because methionine can be transformed into cystine by living tissues.³

It is known that methionine is an essential amino acid, while cysteine and cystine are not (89, 84, 47). Methionine can replace

2. The old literature is extensively quoted in Rothman and Schaaf (69).

3. This has recently been confirmed in wound-healing experiments by Williamson and Fromm (M. B. Williamson and H. J. Fromm, Effect of cystine and methionine on healing of experimental wounds, *Proc. Soc. Exper. Biol. & Med.*, **80**:623-26 1952).

cystine either for growth in general or, more specifically, for the growth of hair (21). How this process may be achieved is shown in Figure 2. The main steps in the conversion are the demethylation of methionine to homocysteine and the formation of an addition product of homocysteine and serine. In view of the low methionine and high cystine

and easily soluble proteins may contain as much cystine as keratin. This is the case with insulin (13 per cent cystine). But it is clear that the salient point is not the amount of cystine but its position in the molecule. It adds to the solidity of the molecule only if its disulfide groups act as a cross-link between two chains.

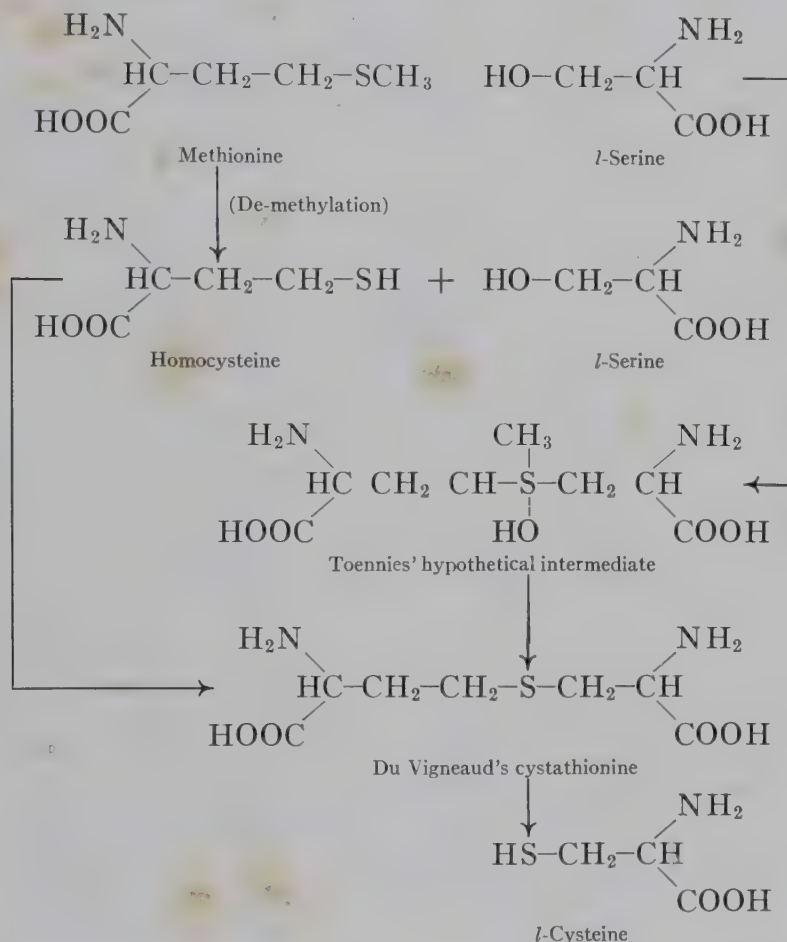


FIG. 2.—The main reactions involved in the conversion of methionine to cystine in vivo (48)

content of keratins, it seems reasonable to assume that all these steps occur in the course of keratinization and that, in the final stage, the sulfur atoms of methionine are incorporated as $-\text{S}-\text{S}-$ cross-linkages into the keratin molecule. Whether all this can occur in the polypeptide chain or only if methionine is free is not known.

The formation of new disulfide cross-linkages satisfactorily explains the resistance of keratins to enzymatic and chemical hydrolysis. It is known that globular, soft,

Insulin can be transformed into a fibrous protein by heating in acid solutions; and Marston (48) thought that this process was reminiscent of keratinization, in which globular proteins of epithelial cells transform into the fibrous state.

C. THE DISAPPEARANCE OF SULFHYDRYL GROUPS AND THE KERATOGENOUS ZONE OF GIROUD

The small number of $-\text{SH}$ groups in keratinized material as compared with that

in cell proteins from which it derives is well established by chemical analysis and by histochemical findings. The early literature on the negative $-SH$ color reaction in keratinized structures, reviewed in my *Chemistry of*

structures, prior to the complete disappearance of the $-SH$ color reaction, there is a zone in which the color reaction is quite intense, more intense than in the cells below (Fig. 3); he called this zone the "phanero-



FIG. 3.- Phanerogenic zone of Giroud. Sodium nitroprusside reaction staining sulfhydryl groups. 1, human sole; 2, human sole—weak reaction of the stratum lucidum; 3, human hair; 4, chestnut from leg of horse; 5, human nail, sagittal section; 6, corn from toe; 7, human nail, transversal section in the level of the nail bed; 8, human nail, transversal section in the level of the nail matrix; 5, 7, and 8 illustrate well the strong reaction of the keratogenous zone. From Giroud and Bulliard (35). (Reproduced by permission of Gaston Doin et Cie.)

the Skin (69), was essentially confirmed in newer work (53). All findings fit well the concept of $-S-S-$ bridge formation from $-SH$ groups in keratinization.⁴

However, a peculiar phenomenon, first described by Giroud (35), has remained without interpretation. Giroud demonstrated that in the development of hard keratin

genic zone," meaning that such a zone occurs in appendages (*phanères*) just prior to their

4. Recent work of Rudall (p. 346) and of Van Scott and Flesch (personal communication) has cast some doubt on the occurrence of disulfide closure in epidermal keratinization as contrasted with keratinization in hair and nails. The assumption has been that disappearance of $-SH$ groups must have some additional, different mechanism.

development but does not occur in epidermal keratinization. Since Giroud's work, however, this zone has been shown to occur also in the epidermis (53), and therefore the term "keratogenous zone" probably is to be preferred.

The sudden appearance, before keratinization sets in, of a relatively great number of sulfhydryl groups can be interpreted in two ways. Either there is a hydrolytic splitting of amino acids (p. 372), with liberation of hidden $-SH$ groups, or hidden $-SH$ groups may get to the surface by the unfolding of coiled chains.

Discussing the possible mechanisms of formation of $-S-S-$ cross-linkages, Marston (48) considered both the possibility that sulfhydryl groups within the polypeptide chain are oxidized in pairs (as shown in Fig. 1) and the possibility of complete hydrolytic breakdown prior to keratin formation; and he considered the latter to be the more probable event, which might explain the existence of a keratogenous zone. But nothing is actually known about this important phase introducing keratinization.

D. THE CATALYTIC EFFECT OF COPPER IN THE FORMATION OF DISULFIDE CROSS-LINKAGES

The catalytic effect of copper in the formation of disulfide cross-linkages is a highly important aspect of keratinization and was studied by Marston (48), whose description is closely followed here. Normally, in the sheep, when hair germ cells mature, little surface is available, and they take up a shape characteristic of minimal surface. In this state they are forced from below toward the conical constriction of the follicle. During this passage outward, the cells are gradually compressed to about one-eighth their original cross-sectional area. They become elongated into the spindle-shaped cortical cells which later, when keratinized, constitute the wool fiber. The basal cells mature and divide approximately every 2 hours, and between 250 and 300 μ of fiber are extruded each day. The histochemical sulfhydryl reaction with sodium nitroprusside is moderate-

ly weak in the basal cells; but at the level where intracellular granules start to appear and microfibrillae are formed, the stain is intense. The strong color reaction of the keratogenous zone extends for approximately 100 μ along the fiber and ceases quite abruptly in the fully keratinized fiber.

In sheep which have been fed on a copper-deficient diet the intense sulfhydryl reaction starts at the same level as in normal animals, but it extends 1,000 μ or more along the fiber. Almost the whole length of the fiber which is imbedded in the skin reacts strongly with the nitroprusside reagent. Only at the level of the follicular orifice does the reaction start to weaken, lessening until it becomes entirely lost in the protruding part.

Parallel to these histochemical findings, Marston has shown that the total sulfur content of wool fibers in copper deficiency is 10–15 per cent lower than normal. The fibers in copper deficiency are weak, as they were after artificial rupture of disulfide bonds. Obviously, there is a slow setting of the fibers, but the α -keratin pattern is present also in copper-deficient fibers; so the setting finally does take place.

On this evidence, Marston has little doubt that oxidative closure of the sulfhydryl residues to disulfide linkages is catalyzed by copper. In the normal integument this oxidation is completed within 8–12 hours. In copper deficiency the process takes 3 days or more for completion, and even then the fiber retains its plasticity in the proximal half of the hair, or at least to the level where the sebaceous glands enter.

Marston emphasized that similar delays in keratinization do not occur in cobalt deficiency and that the effect of copper deficiency is a direct one, and not via oxidative enzymes: there is no change in the status of cytochrome C and cytochrome oxidase of tissues in copper deficiency. He states also that deficiency in vitamin B factors (riboflavin, biotin, pyridoxine, and panthothenic acid), even if decreasing wool production, does not act on the keratinization process proper, as copper does, but acts rather, in-

directly, by modifying the division and maturation of the proliferative cells in the lower strata.

Thus it appears that the effect of copper is quite a specific one. In this connection it is intriguing to recall an earlier statement by Flesch and Rothman (30) that the two most common and general reactions to cutaneous injuries—hyperpigmentation and hyperkeratinization—have in common the transformation of sulfhydryl groups into disulfide groups. One may now add another common feature by stating that in both cases these processes are catalyzed by copper.

In a more recent publication Ellis, Gillespie, and Lindley (25) followed the keratinization process by analyzing wool roots microchemically. They demonstrated that sulfur is present in these roots overwhelmingly in the form of sulfhydryl compounds and not in disulfide linkage. They also found a much higher concentration of copper in the wool root than in the fully formed fiber. They assumed that in some way copper is withdrawn from the root as it is transformed into a fiber. These authors also reported the presence in the wool root of a great variety of enzymes which may promote the transfer of high-energy phosphate bonds required for peptide-bond synthesis. They calculated that conversion of 2 $-SH$ to $-S-S-$ releases energy which conceivably accounts for 1 per cent of peptide bonds in keratin.

E. THE PROBLEM OF ABSOLUTE INCREASE IN SULFUR CONTENT AND THE POSSIBILITY OF HYDROLYTIC DECOMPOSITION

The transformation of methionine into cystine and the oxidation of all sulfhydryl groups to disulfides do not account for the absolute increase in sulfur content that occurs when keratin develops from cellular proteins. According to data of Giroud *et al.* (36)—for instance, in the horn of cattle—the nuous layer contains 1 per cent sulfur per dry weight, while the sulfur content of the keratin is 3.4 per cent of the dry weight. The only available explanation is that, prior to or simultaneously with keratinization, there is hydrolytic splitting-off of

amino acids which do not contain sulfur. If this is the case, the absolute sulfur content, of course, will increase. Again, Unna (81) was the first to postulate hydrolysis in the process of keratinization; but he assumed that cystine is split off in the central, nonkeratinizing part of the cell and that the free cystine migrates to the periphery to be incorporated into the keratinizing membrane. An early, but surprisingly modern, view was that of Sammartino (71). He found in keratins unusually large percentages of tyrosine, tryptophane, cystine, glutamic acid, and arginine and relatively low percentages of aliphatic monoamino acids. This, he concluded, shows that during keratinization the latter acids are split off from the protein molecule and that there is a pseudo-increase in the percentages of the rest.

Evidence that hydrolysis actually takes place during keratinization was presented first by Bolliger (9), who demonstrated the presence of the nonsulfur-containing free amino acids, glutamic, valine, and leucine, in water extracts of hair. He found that 3.5 per cent of the weight of dry, fat-free hair is water-soluble material ("nonkeratins") (10) and that 300 mg. per cent of the weight of hair is accounted for by free amino acids. Similarly, in my laboratory, Sullivan (77) found a great number of free amino acids in cold-water extracts of hair but none of the sulfur-containing amino acids. These findings support the theory that prior to or during the keratinization process hydrolysis takes place and that sulfur-containing fragments are incorporated *in statu nascendi* into the keratins, while other fragments remain in the cell or in the cellular debris as free amino acids.

F. FORMATION OF SALT LINKAGES AND HYDROGEN BONDS

Nothing is known about how these bonds are formed in vivo during keratinization. It is probable, however, at least in the case of hair, that they develop simultaneously with the unfolding of coiled chains into the α -fold pattern. That hydrogen bonds

contribute to the solidity of the epidermal horny layer can be concluded from the keratolytic effect of strong lithium bromide solutions on this layer (p. 359).

G. GLYCOGEN SYNTHESIS OR ACCUMULATION OF GLYCOGEN

Claude Bernard (3, 4) was the first to observe that the soft keratogenous zone of the hoof of the embryonic cow contains abundant glycogen and that it disappears from each cell as the latter becomes keratinized. Unna (82) demonstrated the presence of glycogen in human skin in the "infrabasal horny layer," which is clearly a keratogenous zone; and he observed its disappearance with the onset of keratinization. Recently, his findings were confirmed by Smith and Parkhurst (74), who found glycogen in the stratum granulosum but not in the horny layer.

Most impressive histochemical findings concerning glycogen and hair formation were reported by Montagna, Chase, and Hamilton (58). While there is no glycogen in the actively dividing matrix cells of the bulb, glycogen appears in the next generation of keratin-forming cells of medulla, cortex, and cuticle of hair and in the external sheath. As actual keratinization sets in, the amount of glycogen diminishes and has disappeared completely by the time that keratinization has become complete. The inverse relation between glycogen accumulation and progressive keratinization is unmistakable. For instance, keratinization of cells of the medulla is slower and less complete than in the cortex or cuticle, and, correspondingly, glycogen extends farther distally before disappearing in the medulla. Or there is no glycogen in the inner sheath cells, which are characterized by extremely early and rapid keratinization.

All these findings can be related to observations on the vaginal mucosa, in which keratinization can be provoked by administration of estrogenic hormone. This reaction is associated with the accumulation of glycogen in the mucosal cells (15). What the connection between keratinization and glycogen

synthesis can be, is at present a matter of speculation. Bullough (13) has shown that epithelial mitotic activity of mouse epidermis can be stimulated by starch injection, but also by the injection of phosphates. Possibly high-energy-bond phosphates of adenosine triphosphate are responsible for glycogen synthesis, and the glycogen degradation in keratinization supplies energy for the chemical reaction required therein. In estrogen-stimulated vaginal mucosa, increased lactic acid production was also demonstrated (15).

Bullough (13) has also shown that anything which hinders glycogen synthesis, such as muscular exercise, cold, or phlorizine, suppresses the mitotic activity of epidermal cells. The higher mitosis rate during sleep and rest (p. 597), as compared with that during activity, was interpreted in a similar way. Obviously, depressed mitotic activity involves a decreased rate of keratinization.

Only after completion of this part of the manuscript, did I find the publication of Bradfield (12), who developed in greater detail the idea that glycogen may serve as a source of energy in keratinization. He found that, with the onset of epithelization of experimental wounds in guinea pigs, the new epithelial cells contain large amounts of glycogen. The new epithelial sheath consists of 10–12 layers and has no horny layer. Glycogen is contained in the outer parts, not in the 2 or 3 basal layers. As the outermost cells start to keratinize, they lose their glycogen. After complete healing, when the number of cell layers has been reduced from 10–12 to 4–5, glycogen disappears completely from the newly formed epidermis.

Bradfield's interpretation is as follows: The tall new epithelium, being far from blood vessels, has a poor oxygen supply. It cannot take up oxygen from the outside either, because it is covered tightly by a crust. The cells are compelled to operate with anaerobic glycolysis. Therefore, as they leave the basal layers, they start to store glycogen, which is later used to supply energy for "protein synthesis" (i.e., keratinization). All the glycogen has been used up by

the time that keratinization is complete. Bradfield emphasizes that he has never found glycogen in basal cells.

H. KERATINIZATION: A DENATURATION PROCESS

Astbury and Dickinson (1) regard the unfolding of long coiled polypeptide chains of globular proteins into the β -keratin shape as

the final state of protein denaturation, because protein denaturation always involves the aggregation of peptide chains into parallel bundles, like those in β -keratin. The process involves polymerization ("polymeric degeneration"), decrease in water solubility, and increase in elasticity. In this sense, keratinization certainly can be called a "denaturation process."

II. CELLULAR DECOMPOSITION DURING KERATINIZATION

A. DEHYDRATION

The keratinizing cell loses most of its water. While living cells contain 70–80 per cent water, the water content of human hair was found to be 8.6 per cent (83); of human nails, 10.5 per cent (69); of scales in psoriasis, 8.5 per cent; in ichthyosis, 11.5 per cent (69); and in scarlet fever, 20 per cent (41). Naturally, life cannot go on in this dry state.

TABLE 1*

HOT-WATER EXTRACT OF RABBIT HAIR (9)

	Mg. Per Cent		Mg. Per Cent
Uric acid.....	400	Glycogen.....	400
Other purines...	100	C ₆ H ₁₁ O ₄ N ₃ (citramide?).....	50
Amino acids....	300	Citric acid.....	50
Urea.....	100	Total phenols...	60
Ammonia.....	60		
Pentoses.....	200		

* 3.5 per cent of hair is protein-free solids. Concentrations are expressed in mg. per cent of dry, fat-free weight of hair.

The water content varies greatly, mainly because all horny structures, being on the surface and hygroscopic, may take up moisture from the atmosphere. The seasonal variations in water content of human hair were studied by E. Voit (83). The water liberated from keratinizing cells escapes outward and most probably constitutes part of the insensible water loss (p. 234).

By what mechanisms the dehydration occurs is not understood. But it is possible that it has something to do with the uncoiling of polypeptide chains and the close packing of the fibrillary crystallites.⁵

B. DECOMPOSITION OF CYTOPLASMIC COMPOUNDS

While there is good reason to believe that lipids are, to a great extent, bound in the cell as lipoproteins, they appear separated in the horny structures. The complete decomposition of phospholipids during keratinization is discussed on page 489. Sterols also disintegrate to a slight degree (p. 487).

C. DECOMPOSITION OF NUCLEAR MATERIAL

The disappearance of nuclei from the keratinized cell is well known to morphologists. Their disintegration was observed chemically by Bolliger (9), when he systematically examined the water-soluble compounds in hair of different species. As shown in Table 1, he found 400 mg. per cent uric acid (and 100 mg. per cent other purines) in aqueous hair extract, obviously stemming from the decomposition of nucleoproteins and from oxidation of their purine bases. Similarly, the high ribose and desoxyribose content of these extracts indicates decomposition of the nucleoproteins, particularly because pentoses are present in the hair extract not only in a free state but also as nucleosides. Bolliger recently isolated guanosine, a guanine-pentose complex (11).

Nuclear decomposition is probably secondary to cytoplasmic keratinization. In a pathological type of keratinization, known as "parakeratosis," the keratinization of the cytoplasm may be far advanced, and still the nuclear decomposition is incomplete.

5. In hair the dehydration of cells and sudden rise in birefringence occur at the same level (Mercer, see n. 1, p. 367).

III. PARTICULATE ELEMENTS IN KERATINIZATION

The stratum granulosum and the stratum lucidum are layers interposed between the Malpighian layer and the stratum corneum and can be regarded as "transitional" layers between noncornified and cornified epithelium (66). The cells of these layers contain microscopically visible corpuscular elements—the keratohyalin granules and eleidin droplets. The granules are called "keratohyalin" because they have some connection with keratinization and because they are similar to "hyalin," in so far as they swell in weak alkaline solutions. The term "eleidin" is used to indicate that this material resembles fatty droplets, the Greek word *elaia* meaning "olive oil." The histochemical and staining reactions of these corpuscles have been summarized recently by Smith and Parkhurst (74) (see Table 2).

It could not be decided from microscopic observation whether these particulate bodies derive from the nucleus, from the interfibrillary cytoplasm, or from the fibrillae themselves.

Histochemical solubility tests indicated that they possibly represent proteins or protein decomposition products (82, 40); but actually it was not shown that they are coagulable or precipitable with protein precipitants. Nor is it clear whether or not eleidin has a lipoidic component. The scope of our ignorance was merely broadened when the stratum lucidum was subdivided into pre-eleidinic, eleidinic, and posteleidinic layers and a para-eleidin was described (49, 50, 51), an indication that eleidin is not a well-defined substance. The three layers mentioned above probably correspond with Unna's infrabasal and basal horny layers.

It is most regrettable that, although keratohyalin and eleidin were described more than seventy years ago, still nothing is known about their nature or about their participation in the keratinization process. Certainly, they occur exclusively in keratinizing epithelium. The more they are developed, the stronger and harder the resulting

keratin structures are. If, under the influence of estrogenic hormones or vitamin A deficiency, metaplasia takes place and non-cornifying epithelium is transformed into squamous epithelium, keratohyalin and eleidin will appear in the transitional layers as soon as the epithelium acquires cornifying potentiality. However, it is not quite clear whether or not they are obligatory intermediary products of keratinization. While keratohyalin is easily recognized in cross-sections of adult human skin from any region, eleidin is seen well only in specimens from palms and soles.

In recent times three observations have been reported which may be significant, although they are controversial. First, Smith and Parkhurst (74) reported that keratohyalin and eleidin lose their tingibility and metachromatic stain when the sections are pretreated with ribonuclease. But Lansing and Opdyke (43) could not demonstrate their digestibility with ribonuclease. Thus it remains doubtful whether the particulate matter contains ribonucleic acid. Second, it was shown that keratohyalin stains deeply with unoxidized haematoxylin; and this finding suggested the presence of a bivalent "metallic mordant" in the granules (74). Smith and Parkhurst (74) suspected that the metal salt might be iron or copper, but neither one could be conclusively demonstrated histochemically. In view of the role of copper in keratinization, it would be most interesting to find that copper becomes attached to some proteinic substances in the keratogenous zone, to form a morphologically distinguishable corpuscle. Although it is claimed on morphological grounds that the horny fibers do not arise from these corpuscles (64), this problem is by no means settled. Third, Lansing and Opdyke (43), on the basis of microincineration experiments, are of the opinion that keratohyalin granules are rich in calcium. This observation does not match that of Gans (33), who found no conspicuous accumulation of calcium

in the transitional layers, as shown in his microincineration pictures of the plantar skin.

In Lansing and Opdyke's experiments an extremely well-developed granular layer was produced in the skin of the nipple in guinea pigs by estrogenic stimulation (p. 381). If the stratum granulosum were particularly rich in calcium, one would expect a correspondingly broadened white band in the spodogram at the site of the granular layer.

In the illustrations of the authors, this cannot be recognized clearly.

A fourth finding was the presence of alkaline phosphatase in the transitional layers (74, 43), clearly outside the granules, together with glycogen. Both glycogen and alkaline phosphatase are absent in the horny layer and in other horny materials (74). These findings again point to the possible role of glycogen degradation as a source of energy in keratinization.

TABLE 2 (74)

STAINING REACTIONS OF KERATOHYALIN GRANULES, STRATUM LUCIDUM,
AND STRATUM CORNEUM OF THE SKIN
(Corresponding Structures in Hassall's Corpuscles of Thymus Stain the Same or More Deeply)

Stain and Fixer	Keratohyalin Granules	Stratum Lucidum	Stratum Corneum
Best's carmine alcohol.....	Red-pink	Deep pink	Same
Picrocarmine, Helly's.....	Yellowish-red	Variable yellowish-red	Same
Mallory's c.t. stain alcohol.....	Deep red	Orange-red	Inner blue Outer red
Cajal's triple stain, Helly's.....	Red	Red	Yellow
Cajal's triple stain, Rossman's....	Red to bluish	Red, yellowish-red	Same
Congo red.....	Orange-red	Same	Unstained or variable orange-red
Haematoxylin alcohol			
Mayer's haematoxylin (alum)...	Deep purple	Pale	Same
Unoxidized			
Mallory's.....	Bluish-purple	Tan	Same
After sulphuric acid.....	Very pale	Pale yellow	Same
After oxalic acid.....	No stain	Gray or pale pink	Same, paler
After ribonuclease.....	Bluish-purple	Deep blue	Gradually pale
Macallum's.....	Deep purple	Tan-gray	Paler
After sulphuric acid (Nichol- son's method).....	Pale blue	Pale yellow	Same
Alizarin red S.....	Brownish- or blackish- purple	Orange	Same
After oxalic acid.....	Paler brownish or black- ish
Rinse in acid alc.....	Brownish	Brownish-yellow	Same
Rinse in alkaline alc.....	Violet	Tan	Same
Giemsa's staining solution, Helly's	Bluish-purple, pinkish	Pinkish lavender	Same
Toluidine blue alcohol, Rossman's	Bluish-purple, pinkish	"Green" reaction	Same with super- ficial blue border
Thionine, Rossman's.....	Reddish-purple, pinkish- lavender	"Green" reaction	Same with super- ficial blue border
After ribonuclease.....	No stain	No stain	Same
After hyaluronidase.....	Reddish-purple	Rich blue	Same
Chromation followed by osmication	No color	Brown	Black
Frozen sections, Sudan black B....	No color	Parallel blue-black lines	Same
Silver impregnation.....	No reaction	Parallel blackened lines	Same
Feulgen's technic for thymonucleic acid.....	No reaction	Same	Same
Leucofuchsin technics for carbohy- drates.....	No reaction	Unstained or diffuse and pale	Same
Alkaline phosphatase.....	Faint gray	No reaction	Same

IV. THE DESQUAMATION PROCESS AND ITS ANOMALIES

A. NORMAL SHEDDING

In mammals the horny layer of the epidermis, as contrasted with hair and nails, is continuously shed in invisible small particles. This process involves the disintegration of adherent keratin fibrils into minute microcrystals on the surface. Loosening of fibers can be observed microscopically. While in the deeper strata of the horny layer the fibers are closely packed (stratum compactum), they are looser higher up (stratum disjunctum); but the final shedding cannot be seen under the conventional microscope because of the submicroscopic size of the desquamating particles. Whether the disintegration of keratin fiber aggregates is connected with the breakage of chemical (covalent) bonds is not known. Possibly it is connected with the dehydration.

B. FORMATION OF VISIBLE SCALES

There are two principal pathological forms of aberration in the physiological process of shedding, viz., (a) shedding in micro- or macroscopically visible scales and (b) decrease in the rate of shedding, with the formation of a pathologically thickened horny layer. Morphologically, both processes are called "hyperkeratosis"; but obviously it is desirable to make the above distinction.

There are two different mechanisms whereby visible scales appear on the skin surface.

The first and most common mechanism is an accelerated rate of keratinization, usually caused by accelerated epithelial proliferation. When more cells than normal are formed per unit time, keratinization must proceed faster. Such accelerated keratinization is incomplete, as evidenced by remnants of nuclear structures, by skipping of granule formation (parakeratosis) (45), and by increased loss of water vapor (68). Clinical examples for such desquamation anomalies are exfoliative dermatitis, psoriasis, and postinflammatory scaling (68). By what

mechanism the acceleration of the process inhibits the final degradation of keratin fibers into submicroscopic particles is not understood. There is a good possibility that it has something to do with incomplete dehydration. Unfortunately, nothing is known about the water content of the normally desquamating minute particles, and therefore it is impossible to prove this hypothesis.

The second mechanism whereby visible scales appear on the surface is the cracking of the uppermost horny layer into macroscopically visible plates. In this form of "scaling" there is no acceleration of epithelial proliferation, no acceleration of the keratinization process, no parakeratosis, and no increased insensible water loss. The classical example of this anomaly is ichthyosis vulgaris (68). In ichthyosis there is a thickened horny layer, an indication that desquamation is retarded rather than accelerated, and therefore ichthyosis is regarded as a keratin-separation anomaly (68). Why in ichthyosis the surface layer breaks instead of staying compact and continuing accumulation of horn, as in the case of calluses, is not understood; but it is probable that there is some anomaly of water and lipid demixing in ichthyotic keratinization, because the type of ichthyotic scales is reminiscent of those seen in dry (xerotic) skin (p. 379).

C. INHIBITION OF SHEDDING

Stimulation of the epidermis by moderately injurious chemical and physical agents, particularly mechanical (rubbing, pressure) and actinic stimulation, causes thickening of the horny layer in many instances. This reaction was first studied quantitatively by Miescher (54). He demonstrated that under the effect of repeated ultraviolet radiation the horny layer of the human epidermis thickens quite considerably. This thickening suffices to decrease the absorption of ultraviolet rays to such a degree that it affords considerable protection against any further effect of these rays. The

effect of mild rubbing was studied by Rubin (70) in my laboratory. He obtained thickening of the horny layer by 10–71 per cent (average 38 per cent) after rubbing one spot on the thigh with moderate pressure for 10 minutes daily for 30 days.

It is highly probable that this reactive hyperkeratosis is a very essential factor in the gradually decreasing sensitivity or in the “hardening” of the skin in repeated chemical irritation (65).

It should be emphasized that in these reactions the Malpighian layer is not thickened (70). The reaction is not connected with increased epithelial proliferation but is based solely on a decreased shedding tendency. The most extreme examples of this situation are corns and calluses. The fine mechanism of this phenomenon has not been studied, but, by analogy, the assumption that these nonspecific stimuli induce a more complete keratinization of the epithelial cells than normal seems to be justified. It was pointed out (p. 360) that hard keratin structures (nails, hair, horn), in which keratinization has gone furthest (with the most pronounced formation of disulfide cross-linkages, loss of $-SH$ groups, loss of water, and production of most resistant structures), do not shed at all. If there is a decrease in the ability of the epidermal horny layer to shed, this indicates that it has become more like hard keratins. The term “superkeratinization” is suggested for this phenomenon, which can be defined as a reaction of the epidermis to form more adherent and more keratinized horny lamellae than normal, with a decreased tendency to shed.

The sensitivity of different segments in the surface keratin to nonspecific stimuli is apparently not the same. The closure of sweat-gland pores on nonspecific irritative stimulation, as shown by Shelley (73), is based on superkeratinization of the keratin ring inside the pores (p. 277); at the same time, the surface horny layer is not necessarily thickened. One may conclude that the periporal part of the outer horny layer is particularly sensitive to those stimuli which are likely to cause poral closure. Such a se-

lective sensitivity is well known to occur in the field of keratinization anomalies. Examples are the selective sensitivity of follicular pore keratinization in vitamin A deficiency in man (p. 382) and selective sensitivity of vaginal and nipple epithelium to estrogenic stimulation (p. 381).

The great importance of superkeratinization in pathological events is obvious. Superkeratinization is the introductory phase to precancerous lesions of skin and mucous membranes, such as keratoses, cutaneous horns, and leukoplakias. As long as these precancerous lesions are “quiescent,” they actually do not show any other change but superkeratinization. It is followed only later by atypical cellular proliferation. Long before frank malignancy develops, the first sign of cell mutation is the changed potentiality of epithelial cells to form harder keratins than before. It is remarkable that the stimuli which have been shown by quantitative measurements to cause superkeratinization—ultraviolet light and chronic rubbing—are among the foremost precipitating factors of keratoses and leukoplakias.

D. CHAPPING (“DRY” SKIN, XEROSIS, ASTEATOSIS)

In cold weather and/or after excessive use of soap and water, the skin surface or parts of it in some individuals display superficial cracks in the horny layer, usually in a rhomboidal pattern (Fig. 4). This cracking was thought to be primarily caused by a deficiency in the lipid film on the surface, which is supposed to “lubricate” the horny layer. If this film was too thin or entirely absent, the coherence of horny lamellae might suffer. Hence the name “asteatosis.” This view was held for several reasons, viz., (1) cracked skin feels dry, as if it lacked grease; (2) cracking occurs much more commonly in older people, whose sebaceous-gland secretion is reduced (p. 296); (3) in predisposed persons, seasonal cracking starts in fall or early in winter at atmospheric temperatures at which sebum freezes early and further expulsion of sebum is hindered (p. 291); (4) cracking can be easily counteracted and

eliminated by avoidance of soap and water and application of some greasy material to the skin. The so-called "winter itch," for instance, is essentially a result of such cracking and is easily controlled in this manner.

The time-honored view of the primary role of lipids in maintaining the coherence of the horny layer within the zone of physiological shedding was recently challenged by

neum tends to dry out, since more water can be evaporated from the surface than will reach there by diffusion from below, because of the presence of a water barrier at the base of the horny layer or below it (pp. 32 and 29). The horny layer, however, does not dry beyond the limit of pliability at relative humidities of about 60 per cent and higher, because an equilibrium state exists



FIG. 4.—Chapping

I. H. Blank (6, 7) on the basis of rather simple, but fundamental, experiments. He showed that cornified epithelium obtained from calluses remains soft and pliable as long as it is permitted to hold a minimum amount of water—about 10 mg. per 100 mg. dry weight of keratin—but that it becomes brittle when its water content decreases below this concentration. The presence of lipids in the callus does not keep it flexible if it is allowed to dry out, and the dry callus cannot be softened by adding lipids to it.

Blank (7) found that under most common environmental conditions the stratum cor-

neum does not permit the moisture content of the horny layer to drop below the critical level. Under the low relative humidities of winter weather and heated houses or under the conditions of rapidly moving air in windy weather, the stratum corneum does dry out, and chapped skin can develop because of an undue degree of water loss.

Support of the view that the decrease in relative humidity, by decreasing the water content of the horny layer, is the main factor in chapping has come from some remarkable practical observations of Gaul and Underwood (34), who have given a detailed de-

scription of the manifestations of chapping in man. These authors found a striking correlation between a sudden fall in air moisture, as measured by the dew point, and chapping episodes of the skin. In addition, they found that these episodes were preceded by low barometric pressures followed by sudden rises in barometric pressure, at which time chapping appeared. Wind, as well as the use of water and soap, was found to be a promoting factor. The interpretation of these findings was similar to that of Blank: the low dew point produces marked desiccation of the horny layer. The influence of preceding high moisture and of the use of water and soap was explained by the assumption that preceding hydration of the horny layer promotes its rapid desiccation and its tendency to crack when the humidity drops. Actually, it seems that repeated hydration and drying of horny material breaks its continuity and smoothness and allows the surface to become rough and flaky (8).⁶ Possibly this is due to breakage of hydrogen bonds and/or salt linkages (pp. 357 ff.).

The role of change of atmospheric pressure was thought to be due to fluid accumulation in the epidermis by diffusion from the dermis during low-pressure periods and easier evaporation of this moisture in the period of high pressure; when coupled with a low dew point, this barometric change may contribute to the development of chapping (6). However, one should keep in mind that atmospheric pressure is rapidly equalized within the body.

Prior to Gaul and Underwood (34), Sams (72) called attention to the role of humidity when he found that onset and exacerbations of a number of common skin diseases were associated with a mean monthly temperature of 80° F. and a mean monthly dew point of 70° F. when these factors persisted for 2 weeks or longer.

The beneficial effect of greasing the skin against chappiness was explained by the water-retaining effect of a greasy layer (6). The overwhelming role of the water content of the horny layer in maintaining its pliability has been well illustrated by the work of Blank. The practical implications are enormous. Much work still has to be done to find out whether *in vivo* lipids do not contribute to this pliability. It remains a notorious fact that chapping occurs only at low atmospheric temperatures. Chapping in warm weather is seen only when the skin surface is continuously and energetically defatted by the excessive use of soap and water. In a dry, warm environment, barring artificial defatting, chapping is not seen; yet water evaporation can be maximally rapid. However, in a dry, warm climate the lipid content of the horny layer is high because of the free sebaceous flow (p. 291). Similarly, it is well known that old people predispose to chapping. Nothing is known about changes in hydration of the horny layer with age, but sebaceous excretion is known to be diminished in senescence, in women all over the skin surface (p. 296), in men mainly on the shins.

V. THE EFFECT OF ESTROGENS

Administration of estrogens has a profound effect on the life-processes of epithelial tissues in both sexes. One can differentiate two effects: (a) in squamous stratified epithelium there is enhanced proliferation, keratinization, and, desquamation; and

(b) nonkeratinizing mucous membrane epithelium transforms into stratified squamous epithelium by metaplasia. All these changes are most pronounced in the epithelium of the urogenital tract, but considerable changes

6. In his latest publication Blank reported that the extraction of calluses with lipid solvents followed by extraction with water removes large amounts of a hydrophilic material; thereby the water-holding capacity of the callus greatly decreases. The brittleness and flakiness of the horny layer of skin which

has been washed with water following exposure to alcohol, gasoline, or other organic solvents is caused by this loss of water-holding capacity of the horny layer (I. H. Blank, Further observations on factors which influence the water content of the stratum corneum, *J. Invest. Dermat.*, **21**:259-71, 1953).

also occur in tissues which are not connected with the reproductive system, e.g., skin, nasal mucosa, and gums. In his admirable review article, Zuckerman (90) pointed out that although metaplasia seems to occur even in structures of entodermal origin, this is not quite sure, because possibly the entodermal tissues in question might have been invaded by ectodermal cells during embryonic development.

Wherever stratified epithelium meets glandular mucous epithelium, there is a pro-

tion in response to estrogens were observed in the rhesus monkey (2) on the nape of the neck, over the crown of the head, and, to a lesser degree, over the upper part of the nose, in addition to the perigenital areas. Intense stimulation of the epidermis by estrogens can be observed on the enlarged nipples of guinea pigs (43) (Fig. 5). The enhanced keratinization by estrogens is most marked in exactly the same areas where estrogenic hyperpigmentation is most intense, viz., perigenital areas and nipples (20). Perhaps

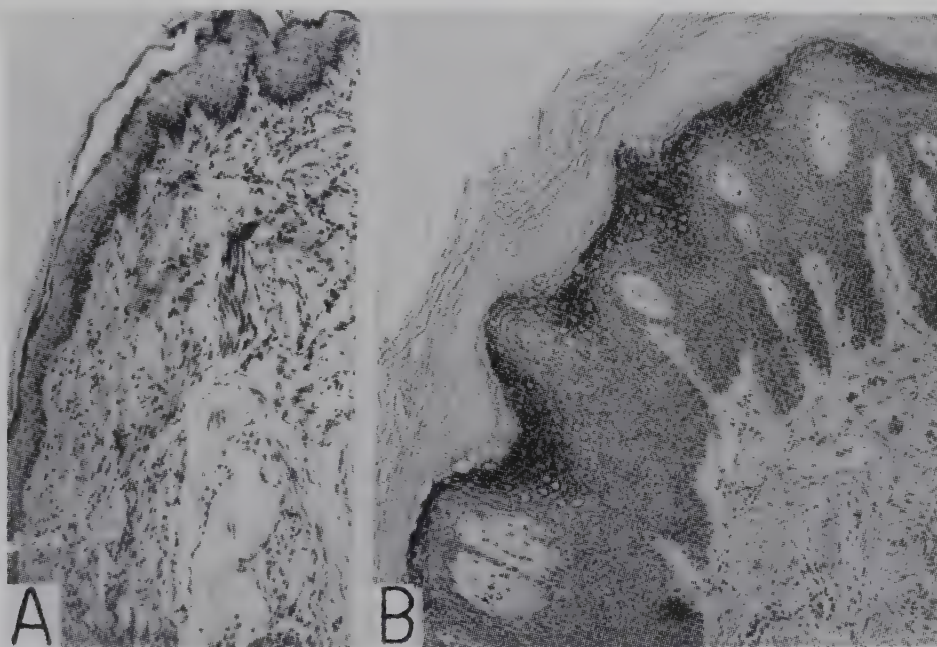


FIG. 5.—*A*, normal guinea-pig nipple; *B*, estrogen-stimulated guinea-pig nipple. From originals of Figs. 1 and 2, A. I. Lansing and D. L. Opdyke, *Anat. Rec.*, **107**:393.

nounced instability of the structures, with a tendency for stratified epithelium to replace cylindrical epithelium even under normal circumstances. These areas are particularly vulnerable to the effects of estrogens (90). Another important point is the auxiliary effect of irritation. Squamous transformation of cylindrical epithelium occurs in many parts of the body as a result of irritation only. Friction was found to be an important factor in the localization of the stratified squamous response in the uterovaginal canal of estrogenized mice (78).

In the skin proper, increased epithelial proliferation, cornification, and desquama-

sulfhydryl groups of melanoblasts and epithelial cells in these areas are more sensitive to stimulation than in similar cells elsewhere, and the elimination of $-SH$ groups may lead to both enhanced pigmentation and keratinization (p. 372). Estrogenic hormone has a pronounced effect upon the growth of feathers (90), and there are some indications that hair cycles in rats and ferrets may be connected with the ovarian cycle (16, 5) (see p. 614).

Not only does the entire thickness of the epidermis respond to estrogenic stimulation, but there are remarkable changes also in the underlying connective tissue, mainly vascu-

lar changes, with increase in water content, erythema, and edema, most pronounced in the perigenital region, the so-called sexual skin, of some monkey species (90). Also, the effect on the nipples is connected with a conspicuous increase in volume. Thus it is not even sure whether the primary attack of estrogens is on epithelial tissue or whether epithelial changes are secondary to vascular and other mesodermal changes; but it is certain that estrogens do not directly modify keratinization. They act by stimulating epithelial cell proliferation and/or by induc-

ing metaplasia in noncornifying basal cells.

Male sex hormone has some action reminiscent of that of estrogens, as shown by Burrows (14). In mice the foreskin detaches from the glans penis after birth. This separation is achieved by transformation of the nonsquamous epithelium of the foreskin into keratinizing epithelium. By castration, the detachment can be prevented. However, when testosterone propionate is given to castrated males, there will be a separation of the prepuce from the glans, by virtue of squamous metaplasia.

VI. THE EFFECT OF VITAMIN A

Like estrogens, vitamin A affects the generative cells of the epithelium and modifies keratinization indirectly. Essentially, vitamin A inhibits the differentiation of stratified squamous epithelium (60) and therefore becomes an "antikeratinizing" factor (39). The reverse "keratinizing effect" of vitamin A deficiency has been known since the early twenties (59, 86, 88). The accumulated data in this field were ably reviewed by Wolbach and Bessey (87).

Analogies between the effects of estrogen and of vitamin A deficiency are striking. They allegedly have an additive effect (60). Both enhance the proliferation of stratified squamous epithelium, and both cause squamous metaplasia of ectodermal, mesodermal, and endodermal mucous membranes, with complete loss of mucous secretion (39). Even the predilectional sites of this "epidermization" by estrogens and by vitamin A deficiency are similar. With particular ease, vitamin A deficiency causes keratinizing metaplasia of the vagina (26), of the seminal vesicles, and of the prostate gland (42), and altogether of all derivatives of the urogenital sinus (85).

Conversely, excess of vitamin A hinders the normal development of stratified epithelium. More than that, if the tissue is continuously flooded with vitamin A, the metaplasia goes in the opposite direction from that of vitamin A deficiency. The epidermal cells become more and more similar to mu-

cous membrane cells (76) (Fig. 6). The most amazing effect of continuously administered vitamin A excess on cutaneous epidermis has been demonstrated recently by Fell and Mellanby (28) in their tissue-culture experiments: cutaneous epidermis is transformed into typical cylindrical mucous epithelium, with secretion of mucous and development of cilia (Fig. 7). This metaplasia is reversible.

The great significance of vitamin A deficiency for dermatological pathology and the selective sensitivity of the epithelium of follicular pores were first recognized by Frazier and Hu in 1931 (31), when they observed, in vitamin A-deficient Chinese, signs of lichen pilaris, a hyperproduction of keratin material in the follicular orifices. This cutaneous disorder was cured by the administration of vitamin A. The sensitivity of the epithelium of follicular orifices could be demonstrated experimentally. In vitamin A-deficient rats, although initially there is a slight generalized hyperkeratosis, soon an increased keratinization of the follicular epithelium is seen (61). The follicles, as in man, become distended and plugged with keratin material. In the final stages, cessation of hair growth, atrophy of the sebaceous glands, and complete disorganization of the hair follicle ensue (61). The orificial epithelium of the fully developed postpubertal hair follicle is more sensitive to the withdrawal of vitamin A than is that of the underdeveloped prepubertal follicle (32).

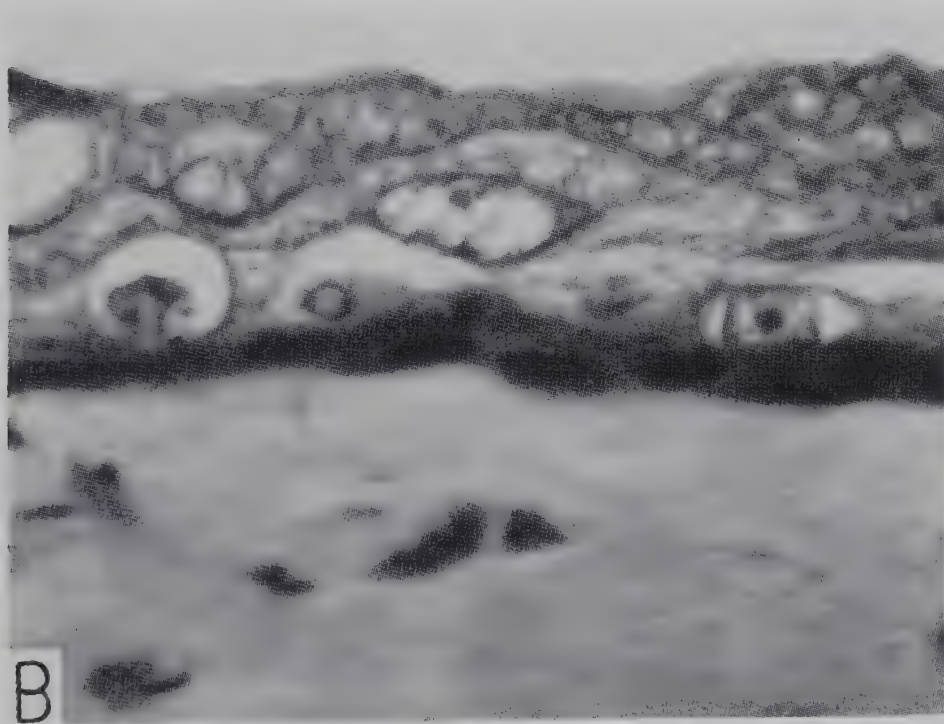
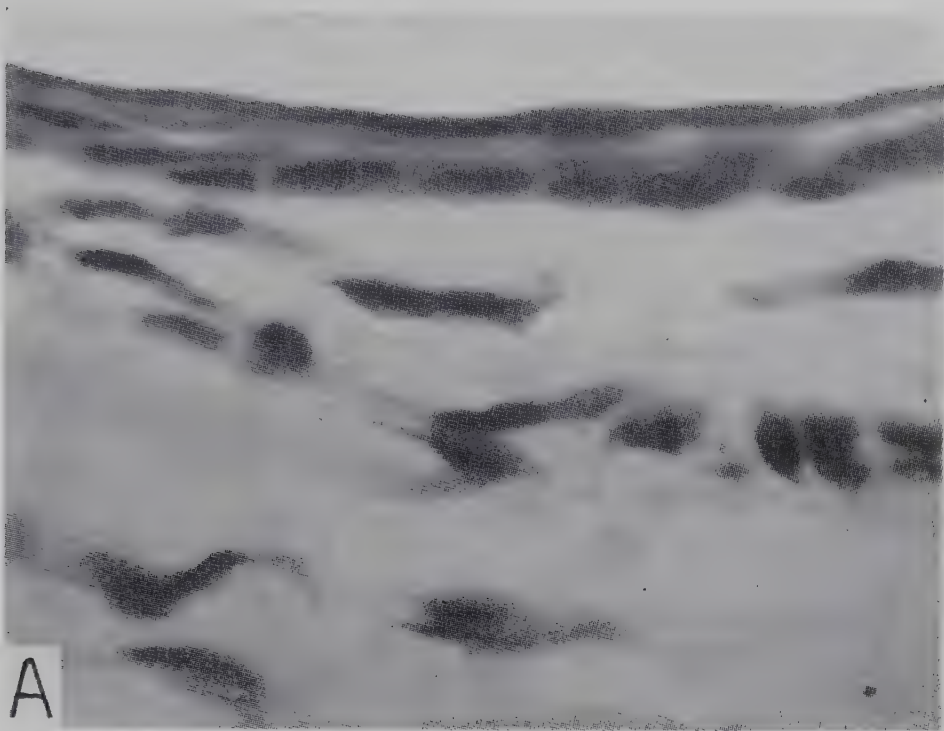


FIG. 6.—*A*, skin from the back of an untreated rat; *B*, state of skin after administration of three doses of 40,000 International Units of vitamin A. Note widening of epidermis, loosening of spinous layer, vacuolation and enlargement of cells. Darkly stained basal layer. From Studer and Frey (76). (Reproduced by permission of Benno Schwabe & Company.)

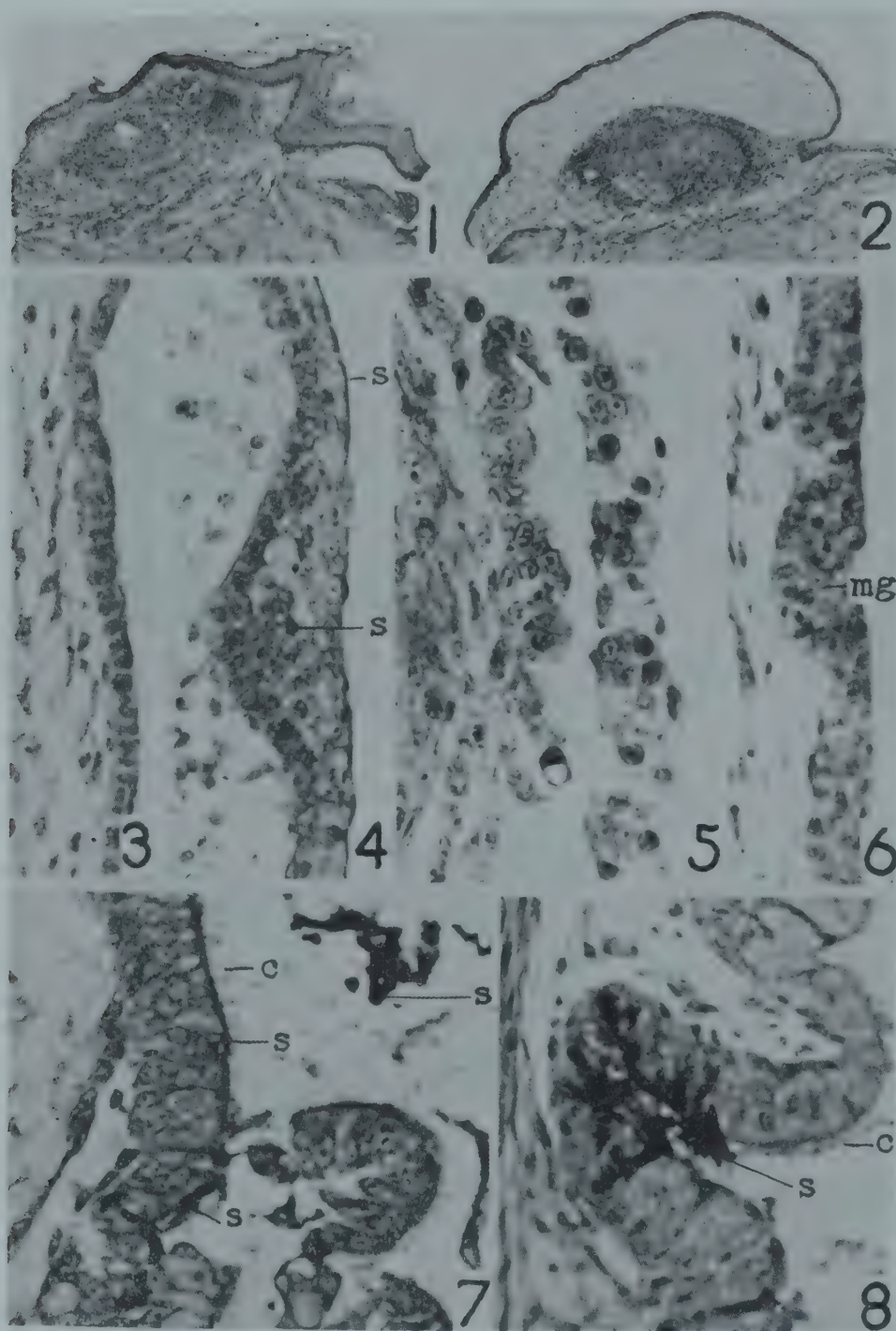


FIG. 7.—1, control explant of a 7-day embryonic skin of chick grown in normal medium for 7 days; watch-glass culture. Note the thick, keratinized squamous epithelium. In the connective tissue there is an outer dermal layer and a more compact inner mass. Cells are migrating into the clot. $\times 48$ (Delafield's haematoxylin; chromotrop).

2, explant from the opposite side of the same embryo grown for 7 days in +A medium; watch-glass culture. Note the absence of keratinization. The connective tissue shows the same two zones as in the control but is rather edematous. $\times 48$ (Delafield's haematoxylin; chromotrop).

3, lateral ectoderm of normal 7-day embryo. It consists of a basal columnar and a superficial flattened layer. $\times 400$ (Azan).

4, skin explant grown in +A medium for 7 days; watch-glass culture. Secretion has just begun (s). The

On experimental grounds it is easy to understand that vitamin A administration or its local application may beneficially influence follicular and nonfollicular hyperkeratotic diseases (such as Darier's disease, pityriasis rubra pilaris, and ichthyosis), even if they do not originate from vitamin A deficiency. Instead of being used as replacement therapy, vitamin A is used on the basis of its pharmacological action. This action consists of suppression of squamous epithelial proliferation and therefore also of keratin formation, in normal as well as in path-

ologically altered skin. Some types of follicular and nonfollicular pathologically increased keratinization and proliferation seem to be more sensitive to excess vitamin A than do others. Whether the keratinization anomaly of follicular orifices in acne vulgaris is influenced beneficially by vitamin A is debatable. Depending on the nature and intensity of the proliferation and keratinization-promoting factor, the dosage of vitamin A required for counteraction may vary greatly and in some cases may be so high as to be beyond the zone of safety.⁷

VII. KERATINIZATION IN VITRO

Since keratinization is an intrinsic feature of the life-cycle of epidermal cells, it is quite natural that it should occur in explants of these cells, notwithstanding their separation from circulation, from the nervous system, and from the action of hormones.

Drew (24) was the first to observe compact areas in epithelial tissue cultures, which, in their arrangement, resembled keratinizing pearls. Erroneously, A. Fischer (29) thought that keratinization in vitro occurs only if the cells are in a condition of poor nutrition. Keratinization in epithelial cultures from chick embryos was observed by Strangeways and Fell (75) and by Fell (27) as a physiological event. Keratinization in explanted human embryonic skin was first reported by Chlopin (18).

The first planned and detailed study on keratinization in vitro was that of Miszurski

in 1937 (55). By using limb buds from the chick embryo, from which the major part of the covering epithelium was removed, he regularly obtained epithelial islets with cen-

7. Flesch has presented evidence that vitamin A has a direct local nonspecific drug effect on epidermal cells when applied in large amounts. The anti-keratinizing effect was shown to be due to the unsaturated double bonds in the molecule; like other unsaturated lipid-soluble compounds, vitamin A interferes with sulfhydryl metabolism; and it was postulated that this was the basis of its antikeratinizing action (P. Flesch, Inhibition of keratin formation with unsaturated compounds. The effect of human sebum on hair growth, *J. Invest. Dermat.*, **19**:353-63, 1952; P. Flesch and S. B. Goldstone, Local depilatory action of unsaturated compounds. The effect of human sebum on hair growth, *J. Invest. Dermat.*, **18**:267-87, 1952). Flesch actually demonstrated that compounds closely related to vitamin A but without vitaminic action have a similar effect, if only they have similar unsaturated double bonds (personal communication; to be published).

epithelium resembles that of the normal nasal mucosa of a 13-day embryo (cf. 6). $\times 400$ (periodic acid-Schiff [P.A.S.] and Meyer's haematoxylin).

5, skin explant after 14 days' cultivation in +A medium; watch-glass culture from same experiment as explant in 4. The ectoderm has formed a mass of irregular cords. $\times 400$ (Azan).

6, normal mucosa from the nasal septum of a 13-day embryo. Note the rudiments of the mucous glands (*mg*). $\times 400$ (P.A.S. and Meyer's haematoxylin).

7, skin explant grown in +A medium for 7 days and then transferred to normal medium for 4 days; watch-glass culture from same experiment as for 4 and 5; note the remarkable differentiation of the ectoderm into ciliated, secretory epithelium (*c*), which closely resembles that of the normal nasal mucosa of an 18-day embryo (cf. 8). $\times 400$ (P.A.S. and Meyer's haematoxylin).

8, normal mucosa from the nasal septum of an 18-day embryo, showing ciliated epithelium and an actively secreting mucous gland. $\times 400$ (P.A.S. and Meyer's haematoxylin). From Fell and Mellanby (28). (Reproduced by permission of the Journal of Physiology.)

tral horn formation. In the outer zones of the islets, the cells had the characteristic features of basal epithelial cells; but toward the inside the cells flattened; and in the center completely keratinized concentric lamellae were present (Fig. 8). The islets were surrounded by a halo of fibroblasts. The

bryo a keratin layer appears on about the eighteenth day, whereas in an explant taken on the sixth day the formation of keratin can be seen as early as 6 days after explantation, i.e., on the twelfth day of life. This could possibly have been the effect of a relative vitamin A deficiency in the cultures



FIG. 8.—Section of a 13-day-old epithelial islet in tissue culture of limb buds from chick embryo. *CT*, connective tissue; *K*, keratinized layer; *EP*, epithelium. From Miszurski (55). (Reproduced by permission of Gustav Fischer Verlag.)

keratinization process in these explants had all the morphological and histochemical characteristics of normal keratinization as seen in the chick embryo and newborn chick, particularly regarding the presence of preleidin and eleidin and tonofibrils and mitochondria. The only difference was a more rapid rate of keratinization in the explant than in the animal, an observation also made on human skin (18). In the chick em-

(28). Miszurski (55) investigated thoroughly the assumption of Fischer that keratinization in explants might be a degenerative phenomenon. He artificially decreased the vitality of his cultures by delaying transfer to new nutrient media, by decreasing environmental temperature, by decreasing the oxygen supply, and, finally, by keeping the cultures in a nitrogen atmosphere. In no case did he succeed in promoting keratiniza-

tion by such measures; and he came to the conclusion that keratinization in epithelial cultures, as in vivo, is the final stage of a process of intrinsic differentiation and not a "degenerative" phenomenon caused by poor nutrition. In the case of rapidly growing epithelium with liquefaction of the nutrient clot, Miszurski observed abnormal keratinization, somewhat reminiscent of parakeratosis, with swollen, nucleated cells.

The findings of Miszurski were confirmed by Litvac (46). She also demonstrated that, in complete analogy with keratinization in vivo, the sulfhydryl color reaction in tissue cultures becomes intensified in the keratoge-

nous zone and disappears when keratinization sets in. Furthermore, histochemical observations suggested that (a) these sulfhydryl groups are protein-bound; (b) there is a great increase in labile sulfur when the cells keratinize (transformation of methionine to cystine?) (p. 369); (c) the addition of cystine does not influence the keratinization process; and (d) young keratin is digested by pepsin, not by trypsin, but mature keratin resists both enzymes.

A modern procedure for studying keratinization in vitro was published by Hanson (38). The recent studies by Fell (28) on vitamin A in vitro have already been mentioned.

BIBLIOGRAPHY

1. ASTBURY, W. T., and DICKINSON, S. The X-ray interpretation of denaturation and the structure of the seed globulins, *Biochem. J.*, **29**:2351-60, 1935.
2. AYKROYD and ZUCKERMAN. Quoted by ZUCKERMAN (90).
3. BERNARD, CLAUDE. *Compt. rend. Acad. d. sc.*, **48**:673, 1859. Quoted by BRADFIELD (12).
4. ———. *Leçons sur les phénomènes de la vie commune aux animaux et aux végétaux*, **2**: 80. Paris, 1878-79. Quoted by BRADFIELD (12).
5. BISSONNETTE, T. H. Relations of hair cycles in ferrets to changes in the anterior hypophysis and to light cycles, *Anat. Rec.*, **63**:159-68, 1935.
6. BLANK, I. H. The relief of the symptom of "dryness." Presentation in the Am. Acad. of Dermat. & Syph. symposium, December 13, 1951.
7. ———. Factors which influence the water content of the stratum corneum, *J. Invest. Dermat.*, **18**:433-40, 1952.
8. ———. Personal communication.
9. BOLLIGER, A. Water extractable constituents of hair, *J. Invest. Dermat.*, **17**:79-84, 1950.
10. BOLLIGER, A., and GROSS, R. Non-keratins: a contribution to the biochemistry of keratinization, *Australian J. Dermat.*, **1**:179-82, 1952.
11. ———. Quantitative studies on some water-soluble organic constituents associated with vertebrate keratin, *Australian J. Exper. Biol. & M. Sc.*, **30**:180-89, 1952.
12. BRADFIELD, J. R. G. Glycogen of vertebrate epidermis, *Nature*, **167**:40-41, 1951.
13. BULLOUGH, W. S. Epidermal mitosis in relation to sugar and phosphates, *Nature*, **163**:680-81, 1949.
14. BURROWS, H. Union and separation of living tissues as influenced by cellular differentiation, *Yale J. Biol. & Med.*, **17**:397-402, 1944.
15. ———. *Biological actions of sex hormones*. Cambridge: At the University Press, 1945.
16. BUTCHER, E. O. The hair cycles in the albino rat, *Anat. Rec.*, **61**:5-20, 1934.
17. CHAMPETIER, G., and LITVAC, A. Structures histologiques et structures moléculaires au cours de la kératinisation épidermique, *Arch. d'anat. micr.*, **35**:65-76, 1940.
18. CHLOPIN, N. Über einige Wachstums- und Differenzierungserscheinungen an der embryonalen menschlichen Epidermis im Explantat, *Arch. f. Entwcklungsmechn. d. Organ.*, **126**:69-89, 1932.
19. COHN, E. J., and EDSALL, J. T. *Proteins, amino acids and peptides as ions and dipolar ions*. New York: Reinhold Publishing Corp., 1943.
20. DAVIS, M. E.; BOYNTON, M. W.; FERGUSON, J. H.; and ROTHMAN, S. Studies on pigmentation of endocrine origin, *J. Clin. Endocrinol.*, **5**:138-46, 1945.
21. DAWBARN, M. Substitution of methionine for cystine in diet of growing rats, *Austra-*

- lian J. *Exper. Biol. & M. Sc.*, **16**:159-68, 1938.
22. DERKSEN, J. C., and HERINGA, G. C. Cornification and tonofibrils of epidermis, *Pol-ska gaz. lek.*, **15**:592-94, 1936.
 23. DERKSEN, J. C.; HERINGA, G. C.; and WEIDINGER, A. On keratin and cornification, *Acta néerl. morph. norm. et path.*, **1**: 31-37, 1937.
 24. DREW, A. H. Growth and differentiation in tissue cultures, *Brit. J. Exper. Path.*, **4**:271-82, 1923.
 25. ELLIS, W. J.; GILLESPIE, J. M.; and LINDELEY, H. Biochemical studies of the wool root, *Nature*, **165**:545-48, 1950.
 26. EVANS, H. M. Effects of inadequate vitamin A on the sexual physiology of the female, *J. Biol. Chem.*, **77**:652-54, 1928.
 27. FELL, H. B. The development in vitro of the isolated otocyst of the embryonic fowl, *Arch. f. exper. Zellforsch.*, **7**:69-81, 1929.
 28. FELL, H. B., and MELLANBY, E. Metaplasia produced in cultures of chick ectoderm by high vitamin A, *J. Physiol.*, **119**: 470-88, 1953.
 29. FISCHER, A. The differentiation and keratinization of epithelium in vitro, *J. Exper. Med.*, **39**:585-87, 1924.
 30. FLESCH, P., and ROTHMAN, S. Role of sulfhydryl compounds in pigmentation, *Science*, **108**:505-6, 1948.
 31. FRAZIER, C. N., and HU, C. K. Cutaneous lesions associated with deficiency in vitamin A in man, *Arch. Int. Med.*, **48**:507-14, 1931.
 32. FRAZIER, C. N.; HU, C. K.; and CHU, F. T. Variations in the cutaneous manifestations of vitamin A deficiency from infancy to puberty, *Arch. Dermat. & Syph.*, **48**:1-14, 1943.
 33. GANS, O. Zur Histo-Topochemie der gesunden und kranken Haut, *Arch. f. Dermat. u. Syph.*, **161**:607-46, 1930.
 34. GAUL, L. E., and UNDERWOOD, G. B. Relation of dewpoint and barometric pressure to chapping of normal skin, *J. Invest. Dermat.*, **19**:9-19, 1952.
 35. GIROUD, A., and BULLIARD, H. La kératinisation de l'épiderme et des phanères. Paris: G. Doin, 1930.
 36. GIROUD, A.; BULLIARD, H.; and LEBLOND, C. P. Les deux types fondamentaux de kératinisation, *Bull. d'histol. appliq. à la physiol.*, **11**:129-44, 1934.
 37. GIROUD, A., and CHAMPETIER, G. Recherches sur les roentgenogrammes des kératines, *Bull. Soc. chim. biol.*, **18**:655-64, 1936.
 38. HANSON, JEAN. Differentiation of mammalian epidermis in tissue cultures, *J. Anat.*, **84**:30-31, 1950.
 39. HARRIS, L. J.; IRMES, J. R. M.; and GRIF-FITH, A. S. Pathogenesis of avitaminosis A; vitamin A as antikeratinising factor, *Lancet*, **223**:614-17, 1932.
 40. HOEPKE, H. Histologische Technik der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **2/1**:498-574. Berlin: J. Springer, 1929.
 41. IONO, Y. Über die chemische Zusammensetzung der menschlichen Epidermis, *J. Biochem.*, **10**:311-23, 1929.
 42. JONGH, S. E. DE. Paradoxe Menformonwirkungen und Vitamin A. *Acta brev. Néerland.*, **4**:70-72, 1934. Quoted by Kongress-zentralbl. f. inn. Med., **78**:530, 1935.
 43. LANSING, A. I., and OPDYKE, D. L. Histological and histochemical studies of the nipples of estrogen-treated guinea pigs with special reference to keratohyalin granules, *Anat. Rec.*, **107**:379-97, 1950.
 44. LAPIERE, S. Les substances à fonction sulfhydrile dans la peau normale et dans divers états pathologiques cutanés, *Arch. belg. de dermat. et de syph.*, **3**:176-87, 1947.
 45. LEVER, W. F. *Histopathology of the skin*. Philadelphia: J. B. Lippincott Co., 1949.
 46. LITVAC, A. Sur la kératinisation épithéliale in vitro, *Arch. d'anat. micr.*, **35**:55-63, 1940.
 47. MARSTON, H. R. Ruminant nutrition, *Ann. Rev. Biochem.*, **8**:557-78, 1939.
 48. ———. Nutrition and wool production. In: *Fibrous proteins: proceedings of a symposium held at the University of Leeds, May, 1946*, pp. 207-14. Bradford: Society of Dyers and Colourists, 1946.
 49. MARTINOTTI, L. Dell'importanza dell'eleidina nei processi patologici della corneificazione, XVII riun. d. soc. ital. di dermat. e sif., Bologna, 1921, pp. 85-90. Quoted in *Zentralbl. f. Haut- u. Geschlechtskr.*, **5**:138.
 50. ———. Ricerche sulle anomalie e le alterazioni del processo della corneificazione nei principali stati morbosi della cute umana, *Gior. ital. d. mal. venereol.*, **62**:681-93, 1921. Quoted in *Zentralbl. f. Haut- u. Geschlechtskr.*, **5**:138.
 51. ———. Ricerche sulle anomalie e le alterazioni del processo della corneificazione nei

- principali stati morbosi della cute umana. Papillomi e verruche, *ibid.*, **64**:810-37, 1923. Quoted in Zentralbl. f. Haut- u. Geschlechtskr., **13**:272.
52. ———. Ricerche sulle anomalie e le alterazioni del processo della corneificazione nei principali stati morbosi della cute umana. Epiteliomi e cisti, *ibid.*, **65**:1353-72, 1924. Quoted in Zentralbl. f. Haut- u. Geschlechtskr., **15**:168.
 53. MESCON, H., and FLESCH, P. Modification of Bennett's method for the histochemical demonstration of free sulfhydryl groups in skin, *J. Invest. Dermat.*, **18**:261-66, 1952.
 54. MIESCHER, G. Das Problem des Lichtschutzes und der Lichtgewöhnung, *Strahlentherapie*, **35**:403-43, 1930.
 55. MISZURSKI, B. Researches on the keratinization of the epithelium in tissue cultures, *Arch. f. exper. Zellforsch.*, **20**:122-39, 1937.
 56. MOERNER, C. T. Eine wohlcharakterisierte organische Schwefelverbindung, erhalten aus Proteinstoffen durch Behandlung derselben mit Salpetersäure, *Ztschr. f. physiol. Chem.*, **93**:175-202, 1914.
 57. MOERNER, K. A. H. Bindung des Schwefels in Proteinen, *Ztschr. f. physiol. Chem.*, **34**:207, 1907.
 58. MONTAGNA, W.; CHASE, H. B.; and HAMILTON, J. B. The distribution of glycogen and lipids in human skin, *J. Invest. Dermat.*, **17**:147-57, 1951.
 59. MORI, S. Changes in para-ocular glands which follow administration of diets low in fat-soluble A: effect of same diets on salivary glands and mucosa of larynx and trachea, *Bull. Johns Hopkins Hosp.*, **33**:351-59, 1922.
 60. MORITZ, WALTER. Das Vitamin A als Differenzierungshemmer des gesamten Epithels, *Ztschr. f. Anat. u. Entwcklngsgesch.*, **112**:271-303, 1943.
 61. MOULT, F. H. Histopathology of rat skin in avitaminosis A, *Arch. Dermat. & Syph.*, **47**:768-77, 1943.
 62. MUELLER, J. H. A new sulfur-containing amino-acid isolated from casein, *Proc. Soc. Exper. Biol. & Med.*, **19**:161-63, 1922.
 63. ———. New sulfur-containing amino-acid isolated from hydrolytic products of protein, *J. Biol. Chem.*, **56**:157-69, 1923.
 64. PERNKOPF, E., and PATZELT, V. Anatomie und Histologie der Haut. In: ARZT and ZIELER, *Die Haut- u. Geschlechtskr.*, **1**:1-140. Berlin and Vienna: Urban & Schwarzenberg, 1934.
 65. ROTHMAN, S. Behandlung des Lupus vulgaris mit Kupferdermasan, *Ztschr. f. Tuberk.*, **36**:342-46, 1922.
 66. ———. Resorption durch die Haut. In: BETHE, *Handb. d. norm. u. path. Physiol.*, **4**:107-51. Berlin: J. Springer, 1929.
 67. ———. Discussion of MESCON and FLESCH (53).
 68. ROTHMAN, S., and FELSHER, Z. Insensible perspiration and keratinization process, *Proc. Soc. Exper. Biol. & Med.*, **56**:139-41, 1944. See also: *J. Invest. Dermat.*, **6**:271-78, 1945.
 69. ROTHMAN, S., and SCHAAF, F. Chemie der Haut. In: JADASSOHN, *Handb. d. Haut- u. Geschlechtskr.*, **1/2**:161-377. Berlin: J. Springer, 1929.
 70. RUBIN, L. Hyperkeratosis in response to mechanical stimulation, *J. Invest. Dermat.*, **13**:313-15, 1949.
 71. SAMMARTINO, U. Zur Kenntnis der Keratinisation, *Biochem. Ztschr.*, **133**:476-86, 1922.
 72. SAMS, W. M. Humidity. Its relation to problems in dermatology, *South. M. J.*, **44**:140-47, 1951.
 73. SHELLEY, W. B., and HORVATH, P. N. Experimental miliaria in man. III. Production of miliaria rubra (prickly heat), *J. Invest. Dermat.*, **14**:193-204, 1950.
 74. SMITH, C., and PARKURST, H. T. Studies on the thymus of the mammal. II. A comparison of the staining properties of Hassal's corpuscles and of thick skin of the guinea pig, *Anat. Rec.*, **103**:649-73, 1949.
 75. STRANGEWAYS, T. S. P., and FELL, H. B. Experimental studies on the differentiation of embryonic tissue growing in vivo and in vitro. I. The development of the undifferentiated limb-bud (a) when subcutaneously grafted into the post-embryonic chick, and (b) when cultivated in vitro, *Proc. Roy. Soc. London, s.B.*, **99**:340-66, 1926.
 76. STUDER, A., and FREY, J. R. Ueber Hautveränderungen der Ratte nach grossen oralen Dosen von Vitamin A, *Schweiz. med. Wchnschr.*, **79**:382-84, 1949.
 77. SULLIVAN, M. B. Personal communication.
 78. SUNTZEFF, V.; BURNS, E. L.; MOSKOP, M.; and LOEB, L. On the proliferative changes taking place in the epithelium of vagina and

- cervix of mice with advancing age and under the influence of experimentally administered estrogenic hormones, *Am. J. Cancer*, **32**:256-89, 1938.
79. SZODORAY, L. Beiträge zur Eiweisstruktur des Hautepithels, *Arch. f. Dermat. u. Syph.*, **159**:605-10, 1930.
80. TOENNIES, G. Sulfur-containing amino-acid methionine; review of present state of knowledge, *Growth*, **1**:337-70, 1937.
81. UNNA, P. G., and GOLODETZ, L. Biochemie der Haut. In: OPPENHEIMER, Handb. d. Biochem., suppl. vol., p. 327. 1st ed. Jena: G. Fischer, 1903.
82. UNNA, P. G., and SCHUMACHER, J. Lebensvorgänge in der Haut der Menschen und der Tiere. Leipzig and Vienna: F. Deuticke, 1925.
83. VOIT, E. Über die Grösse der Erneuerung der Horngebilde beim Menschen. I. Die Haare, *Ztschr. f. Biol.*, **90**:508-24, 1930.
84. WHITE, A., and BEACH, E. F. Role of cystine, methionine, and homocystine in nutrition of rat, *J. Biol. Chem.*, **122**:219-26, 1937.
85. WILSON, J. G., and WARKANY, J. Epithelial keratinization as evidence of fetal vitamin A deficiency, *Proc. Soc. Exper. Biol. & Med.*, **64**:419-22, 1947.
86. WILSON, J. R., and DU BOIS, R. O. Keratomalacia in infant, with postmortem examination, *Australian J. Dis. Child.*, **26**:431-46, 1923.
87. WOLBACH, S. B., and BESSEY, D. A. Tissue changes in vitamin deficiencies, *Physiol. Rev.*, **22**:233-89, 1942.
88. WOLBACH, S. B., and HOWE, P. R. Vitamin A deficiency in the guinea-pig, *Arch. Path. & Lab. Med.*, **5**:239-53, 1928.
89. WOMACK, M.; KEMMERER, K. S.; and ROSE, W. C. Relation of cystine and methionine to growth, *J. Biol. Chem.*, **121**:403-10, 1937.
90. ZUCKERMAN, S. The histogenesis of tissues sensitive to oestrogens, *Biol. Rev.*, **15**:231-71, 1940.

CHAPTER 17

Collagen, Reticulin, and Elastin

By ZACHARY FELSHER

I. X-RAY DIFFRACTION	391
II. COLLAGEN	393
A. General Properties	393
B. Purification	394
C. Amino Acid Content	395
D. Molecular Weight	396
E. Carbohydrate Content	396
F. Action of Proteolytic Enzymes	397
G. Effect of Hormones on Collagen	398
H. Structure	399
I. Birefringence	403
J. Cross-Linkages	404
K. Gelatin Formation and Shrinkage of Collagen	404
L. Swelling Properties	405
M. Isoelectric Point	407
N. Electron Microscopy	407
O. Procollagen	409
III. RETICULIN	409
IV. ELASTIN	410

COLLAGEN, reticulin, and elastin, like keratin, belong to the group of fibrous proteins made up of elongated chains of α -amino acids joined together by the peptide linkage, the chains themselves being held in more or less parallel alignment by various cross-linkages (Fig. 1), differing from those

in keratin by type and number. Mainly on the basis of differences in the X-ray spectrogram, Astbury has separated, within the group of fibrous proteins, collagen from the keratin-myosin-epidermin-fibrinogen group. *Gross* fibers of the latter group are more elastic than those of collagen (3, 93).

I. X-RAY DIFFRACTION

In crystals every atom or molecule has a fixed position with reference to the three dimensions of space. Crystalline aggregations of molecules or structures approaching the crystalline state can be analyzed by the X-ray diffraction method. When an X-ray beam is passed through material having a periodic structure, the beam is diffracted in

a characteristic fashion according to the dimensions of the crystalline lattice and the degree of crystallinity (1). The diffracted beam can then be photographed. Figure 2 illustrates how an X-ray beam, thrown perpendicular to the main axes of a theoretical fibrous protein, produced a diffraction pattern on a film held on the opposite side. A

fibrous protein diffracts a given wave length of the incident X-ray beam in various directions, depending on its molecular arrangement and spacing, producing a pattern of spots, arcs, or halos on the photographic film (Fig. 3). The radius of the rings gives the over-all length of the repeating structural unit. The smaller the unit, the larger

"backbone" of the fiber are probably coiled or folded. That such is the case in collagen, and especially elastin, is very probable.

There are two main cross-sectional dimensions in the X-ray diagram. One measures 4.5 Å. This dimension is approximately the same for all proteins and is the lateral

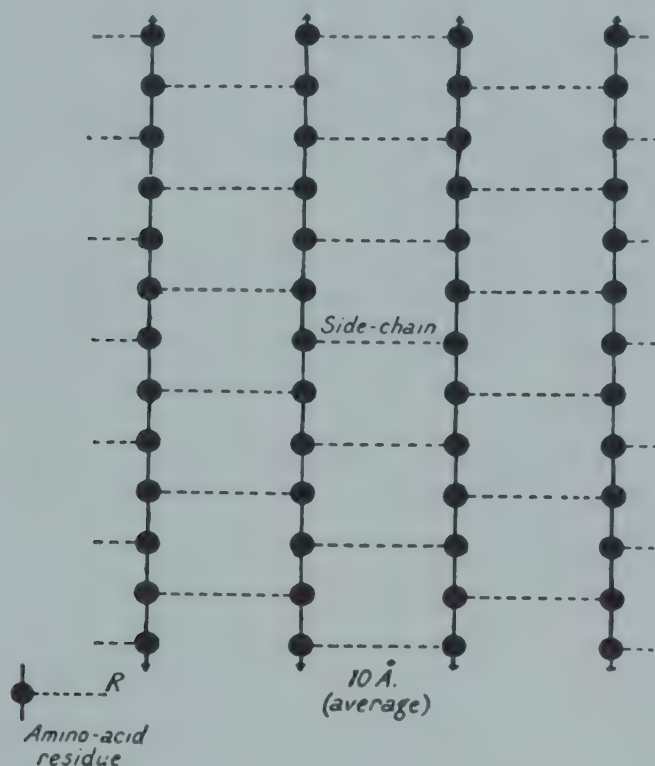


FIG. 1.—Diagrammatic representation of the structure of fibrous proteins as it occurs in β -keratin, according to Astbury. A grid of fully extended polypeptide chains. From McLaughlin and Theis (85). (Reproduced by permission of Reinhold Publishing Corporation.)

the ring. The relative position on the ring of the arcs or spots shows the degree of orientation within the structure (78). In the protein of Figure 2 the polypeptide chains are in a fully extended position; and in this case the distance between identical atomic groups which are repeated in the long axis is about 7 Å, which includes two amino acid residues. The distance, then, for one amino acid residue is 3.5 Å. If this distance is less than 3.5 Å, then it may be assumed that the amino acid residues of the long axis or

distance between parallel backbones, formed probably by the hydrogen bond (p. 357), which acts as one type of cohesive force holding the backbones together. The second cross-sectional dimension is the one by which the projections of the various lateral side chains of the main axis separate the main longitudinal chains of the protein. It varies from protein to protein, but measures about 10 Å in collagen. As the moisture content of the fiber increases, this dimension enlarges.

II. COLLAGEN

A. GENERAL PROPERTIES

Collagen, as well as elastin and reticulin, belongs to the group of proteins which in the past were classified as "scleroproteins." They generally are insoluble in organic solvents, cold water, dilute acids, alkalies, and salt solutions (78, 64).

pH some swelling takes place. This ability possibly plays an important role in the various pathological changes that collagen can undergo in the skin. Peptic enzymes can digest native collagen fibers; but if trypsin attacks them at all, it does so with difficulty.

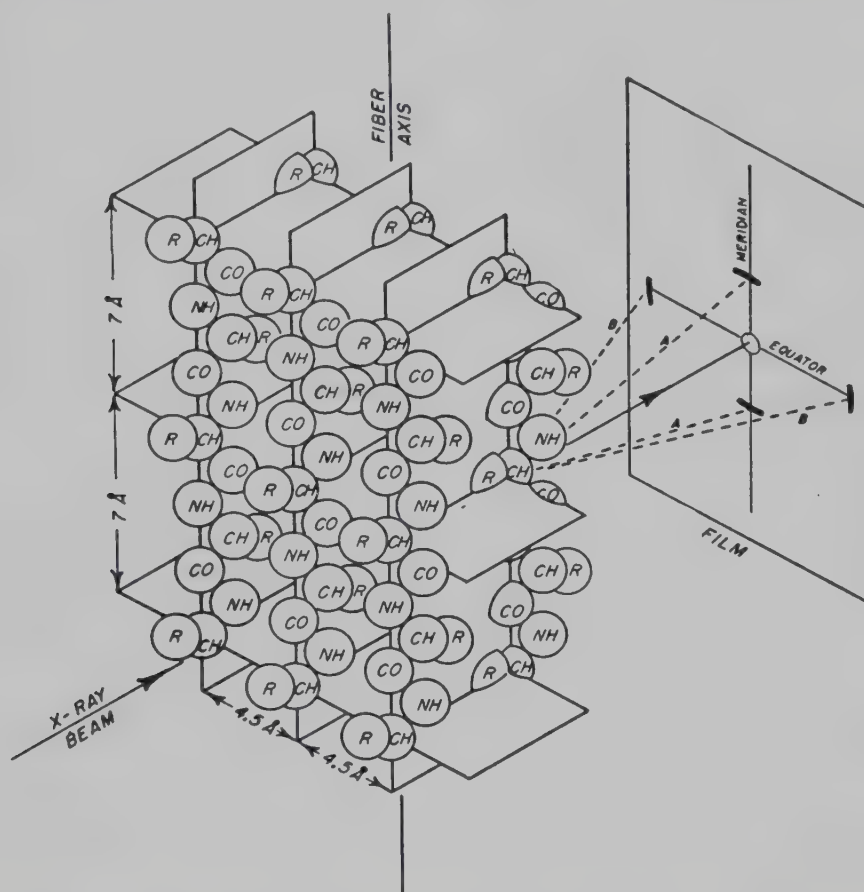


FIG. 2.—Illustrating the formation of a fiber diagram by the diffraction of parallel, monochromatic X-rays by an idealized protein structure composed of fully extended polypeptide chains, when the X-ray beam is perpendicular to the fiber axis. The diffraction may be considered a reflection from hypothetical planes passing through like atom groups. The angle of incidence of the X-ray beam with the planes and the distance apart of the latter must satisfy Bragg's law relating these quantities to the X-ray wave length, in order for "reflection" to occur. *A*, beams reflected from transverse planes, producing diffraction spots on the meridian of the film; *B*, beams reflected from parallel planes, producing spots on the equator. From McLaughlin and Theis (85). (Reproduced by permission of Reinhold Publishing Corporation.)

When collagen fibers are heated in water at gradually increasing temperatures, they first shrink and then are converted to gelatin. In acids, alkalies, and neutral salt solutions, collagen shows a very large capacity to swell. Even in distilled water at neutral

There is little evidence that there are different collagens in the mammalian organism, as are found with the keratins (85). The chemical composition and physical properties of mammalian collagen from different sources are identical, and under the

electron microscope samples of collagen from different tissues look very much the same (p. 407). There is, however, a remarkable exception. Collagen from the tail of the white rat is soluble in dilute 0.4 per cent

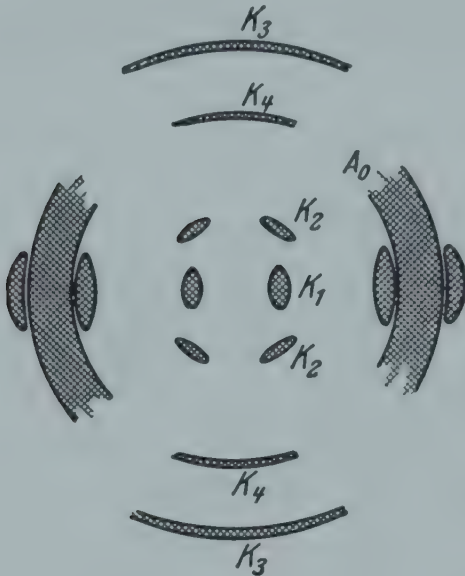


FIG. 3.—Diagrammatic representation of the X-ray pattern of collagen or of stretched gelatin. Fiber axis vertical. From McLaughlin and Theis (85). (Reproduced by permission of Reinhold Publishing Corporation.)

The metabolic activity of collagen seems to be low. Neuberger, Perrone, and Slack (106, 96) fed radioactive glycine to rats and found an extremely low radioactivity of the glycine obtained from tendon collagen, an indication that there is little collagen synthesis going on. Although Danielli and his co-workers found alkaline phosphatase activity associated with collagen formation (28, 34), later investigators have not been able to confirm this (109, 40, 118).

Gelatin does not act as an antigen, and apparently collagen also possesses no antigenicity. Waksman and Mason could not demonstrate the formation of antibodies when rabbits were treated with collagen of human origin (122). Complement-fixation and precipitation tests, likewise, failed to show antibodies to human collagen in patients with lupus erythematoses, dermatomyositis, scleroderma, and rheumatic fever.

B. PURIFICATION

Owing to the difficulty in obtaining a pure collagen preparation in past years, most of the older data pertaining to its amino acid content was obtained from the analysis of

TABLE 1
ANALYSES OF CONNECTIVE-TISSUE FIBERS* (20)

	Total N (Gm. Per Cent)	Amide-N (Mmol/Gm)	Amino-N (Mmol/Gm)
Collagen (from ox hide).....	18.6	0.66	0.46
Collagen (sheep skin, alkali-treated).....	17.3	.35	.35
Elastin (from lig. nuchae).....	17.0	.20	0.07
Reticular tissue (from fat of ox).....	16.1	.50
Reticular tissue (from lymph node).....	16.1	0.58

* Moisture- and ash-free basis.

acetic acid, while no other collagen has this property (94, 121). Fish collagen differs from mammalian collagen by shrinking at a lower temperature and by a greater susceptibility to enzymatic hydrolysis (51).

Collagen makes up about 71.9 per cent of the weight of dry, fat-free human skin. Table 1 shows the total N, amide-N, and amino-N content of collagen, elastin, and reticular tissue (20).

gelatin, the protein derived from collagen by heating in water. In more recent years, methods for the purification of collagen have been improved. Yet even with these methods we cannot be sure that any of the chemical treatments employed to separate collagen from other proteins of connective tissues, particularly mucoproteins, and other conjugated proteins in close association with it have not changed its chemical composi-

tion. The problem of preparing a "pure" collagen remains a difficult task.

One of the often used methods of obtaining a "purified" collagen was that of Highberger (54). Proceeding on the hypothesis that 0.1 per cent trypsin in $\frac{1}{2}$ per cent calcium carbonate at a temperature not higher than 35° C. will not effect collagen, Highberger used this solution to eliminate the soluble proteins and elastin in the corium of the skin. Lipids were removed by extraction with acetone and alcohol, and mucoids were dissolved out by extraction with half-saturated calcium hydroxide, again on the assumption that such treatment does not attack collagen.

Loofbourow, Gould, and Sizer (79) measured the purity of collagen preparations by ultraviolet absorption spectra (see below). They obtained the "purest" collagen by extracting tendon in 10 per cent sodium chloride and M/15 disodium hydrogen phosphate after the method of Bergmann and Stein (14), followed by digestion with trypsin at pH 7.0 at 37° C. (79).

Cassel and Kanagy (23) studied several methods of purification of collagen in steer hide and followed these by nitrogen determinations, electrometric titration analysis, and electrophoretic measurements. They obtained evidence that trypsin inevitably—at least mildly—modified collagen so that subsequent treatment of it resulted in degradation of the product. This was shown by large differences in values for soluble nitrogenous material in the lime extractions for the trypsin- and nontrypsin-treated collagen samples. Cassel and Kanagy found that if the lower part of the corium, excluding the pars papillaris, is used to obtain collagen, the trypsin treatment could be omitted, since only traces of elastin are present there. In their method 5 per cent sodium chloride is used to dissolve the soluble proteins; subsequently, the lime extraction is carried out as in Highberger's method to dissolve mucoids. Cassel and Kanagy further set up certain specifications for a "pure" collagen as follows: (a) an ash content below 0.1 per cent; (b) an isoelectric point between 6.0 and

7.0 (p. 407); (c) a total nitrogen content of 17.8–18.1 per cent; and (d) an amide-nitrogen of 3.8 per cent of the total nitrogen after hydrolysis in 0.1 N HCl for 20 hours.

Bowes and Kenten (19) criticized the use of both alkali and enzyme in attempts to purify collagen. In their method, only the lower part of the corium was used, and this was extracted with 10 per cent sodium chloride alone, to dissolve out the soluble proteins. They considered that only collagen and a few reticular and elastic fibers were left behind.

Several methods have been developed to determine the percentage of collagen and elastin in various tissues (80, 98, 32). Chung K. Ma (82) found a marked increase in the amount of collagen and elastin in skin of mice where carcinoma was induced by application of methylcholanthrene.

C. AMINO ACID CONTENT

The most complete analyses of the amino acid composition of collagen are given in Table 2, as compiled by Bowes and Kenten from their own work and that of other investigators (19). The total of 99.8 per cent nitrogen accounts for all the amino acids. According to the above authors, it is unlikely that any other amino acids will be found in the collagen molecule. The data, of course, can have absolute validity only if the material used for the analyses can be regarded as "pure" collagen without admixture of extraneous material.

In any case, all available data indicate that collagen is characterized by a high content of glycine, proline, and hydroxyproline; a very low content of tyrosine, methionine, and histidine; and the absence of cystine and tryptophane. Loofbourow, Gould, and Sizer (79) found no evidence of the presence of tyrosine by the use of ultraviolet spectrographic analysis of collagen.

Collagens from various mammalian sources seem to have identical or, at least, very similar amino acid composition (97). Collagen from the fish and turtle, however, differ in their content of one or more of the three amino acids, methionine, serine, and

threonine, as compared to the tissues of higher vertebrates (97).

Stubbings and Theis (117) maintained that there are chemical differences in the collagen of the papillary and reticular layer of the skin. They obtained analytic evidence that collagen fibers from the papillary layer of animal skin contained about 33 per cent

threonine, and hydroxyproline; and (*d*) non-polar groups of hydrocarbon character.

D. MOLECULAR WEIGHT

Bowes and Kenten (19) calculated the minimum molecular weight of collagen to be 39,000 from their amino acid data. Bear (10), from data based on X-ray diffraction and electron microscopic findings, has calculated the molecular weight of collagen to be between 63,000 and 65,000 (p. 401).

No free α -amino or imino groups have thus far been detected in collagen (18). Bowes and Moss (21) have suggested that this may indicate a very large molecular weight. However, failure to detect such end-groups could also be explained by a cyclic structure of the molecule, or perhaps the end-group is combined with carbohydrate.

E. CARBOHYDRATE CONTENT

In 1935 Grassmann and Schleich (42) reported that, in collagen of animal skin, glucose and galactose are tightly bound to the protein moiety. They claimed that 1 mol glucose and 1 mol galactose were bound to a collagen molecule of the approximate molecular weight of 34,500. Beek, however (12), reported that the carbohydrate attached to the collagen molecule could not be fermented by yeast, and he considered the possibility that these carbohydrates could be levorotatory glucose and galactose. These stereoisomers are not attacked by yeast. It has already been mentioned that failure to detect free α -amino or imino groups in collagen could theoretically be explained by their being combined with carbohydrate (21). Bowes (18) has stated that hyaluronic acid is probably free in the skin, while chondroitin sulfate is combined in some way with collagen. The amount of chondroitin sulfate estimated in collagen from its hexosamine and chondrosamine content on paper chromatograms corresponded approximately to 1 mol of chondroitin sulfate per 1 collagen unit of 80,000; that is, roughly twice the minimum molecular weight of collagen (39,000) (21). Ein-

TABLE 2

COMPOSITION OF COLLAGEN (19)

	N as Per Cent Protein-N	Gm/100 Gm Collagen
Total N.....		18.6
Amino-N.....	2.5	0.46
Glycine.....	26.3	26.2
Alanine.....	8.0	9.5
Leucine.....	3.2	5.6
Isoleucine.....		
Valine.....	2.2	3.4
Phenylalanine.....	1.9	4.2
Tyrosine.....	0.6	1.4
Tryptophane.....	0.0	0.0
Serine.....	2.5	3.4
Threonine.....	1.5	2.4
Cystine.....	0.0	0.0
Methionine.....	0.4	0.8
Proline.....	9.9	15.1
Hydroxyproline.....	8.0	14.0
Lysine.....	4.7	4.5
Hydroxylysine.....	1.2	1.3
Arginine.....	15.3	8.8
Histidine.....	1.2	0.8
Aspartic acid.....	3.6	6.3
Glutamic acid.....	5.8	11.3
Amide-N.....	3.5	0.66
Total found.....	99.8	

containing hydroxyl groups, such as serine, less lysine than did fibers from the deeper layers. Previously, Highberger (55) had found no significant difference in the lysine content of collagen fibers from the upper tenth of the corium and those from the corium as a whole.

Bolduan, Salo, and Bear (17) have listed the side chains of the collagen molecule as follows: (*a*) basic groups of lysine, hydroxylysine, arginine, and histidine residues; (*b*) acidic groups of aspartic acid, together with glutamic acid residues; (*c*) other polar types

binder and Schubert (31) found no evidence that "purified" collagen could fix chondroitin sulfate at neutral pH in vitro. Highberger, Gross, and Schmitt (57) studied the precipitation of fibrils from solutions of dissolved rat-tail tendon in dilute acetic acid. If "purified" mucoprotein was added to these solutions in the ratio of 1:1,000, typical collagen fibrils were precipitated. The authors suggest that mucoprotein may be involved in the formation of collagen fibers in vivo.¹

F. ACTION OF PROTEOLYTIC ENZYMES

As mentioned before, pepsin digests collagen readily. Tryptic enzymes, if they attack collagen at all, do so with difficulty. Originally, Marriott (88) claimed that if collagen fibers are subjected to tryptic digestion at a pH of 7.8 in a suspension of calcium carbonate and at a temperature no higher than 35° C., no digestion took place. Highberger's method of preparing pure collagen was based on this claim. Marriott found that at 40° C. definite digestion occurred. If collagen was pretreated with acids, bases, salts, or heating, trypsin attacked it much more readily. The same was true if the collagen fibers were cut or torn. Sizer (114) found that although native tendon was refractory to the action of the enzyme, collagen fibrils of the same tendon, cut into pieces less than 1 mm., were digested by trypsin. Treatment with sodium chloride and other salts greatly facilitated the process. Thoreaux (120) showed that although trypsin did not grossly attack collagen fibers, they became abnormally soluble in water at 68° C. after treatment. It seemed possible that trypsin might induce some mild change in the collagen molecule, such as a weakening of the intermicellar and intramicellar valency forces that stabilize the molecule in its native state. Because of probable alteration by trypsin, Cassel and Kanagy (23) objected to the use of this enzyme in the purification process of collagen. Trypsin, however, has not been found

to affect the gross structure of collagen fibrils under the electron microscope (107, 44).

In 1937 Maschmann (90) found that culture filtrates of *Clostridium welchii* liquefied gelatin and disintegrated collagen. He attempted to purify the active principle and suggested the name "collagenase" for it. In 1945 Macfarlane and MacLennan (84) found that the collagen framework of muscle was disintegrated when *Cl. welchii*, type A, culture filtrates were injected into rabbits. Collagen fibers from human tendon were lysed by the same filtrate. Oakley, Warrack, and van Heyningen (101) showed collagenase to be distinct from other toxins of *Cl. welchii* and from hyaluronidase. They called the collagenase the "K toxin." Bidwell and van Heyningen (15) succeeded in "purifying" the K toxin and showed that it appeared to attack only collagen and gelatin; but they were unable to determine which type of linkage it attacked. The "collagenase" enzyme dissolved collagen fibers rather easily, but the production of free amino groups was very slow. More recently, however, Brisou and Milhade (22) found that *Cl. welchii* filtrates had no effect on beef tendon or beef skin collagen prepared by the method of Bowes and Kenten (19). Gersh and Catchpole (39) also could demonstrate no effect of a "collagenase" preparation² on purified collagen but showed that it removed polysaccharide substances from connective tissue. Stoughton and Lorincz (116), using the same preparation on acetone-fixed sections of skin, found the collagen morphologically intact but changed in staining qualities and easily solubilized in hot water.

Palitz and Brunner (103) reported that the injection of hyaluronidase into patches of localized myxedema not only decreased the mucoid substances but also seemed to destroy collagen fibers.

Hobson (58) found an enzyme in sterile excreta of the blowfly larvae which digested collagen and elastin in alkaline solution, but not keratin.

1. See sections on procollagen (pp. 409 and 447).

2. Obtained from Lederle Laboratories.

G. EFFECT OF HORMONES ON COLLAGEN

The effect of various hormones on the skin has been studied experimentally in the laboratory animal. Hooker and Pfeiffer (61) found that the administration of estradiol benzoate to rats resulted in thinning of both the epidermis and the corium. If testosterone propionate was given at the same

the skin in these animals was about one-third that of normal. If growth hormone was given to the same animals, the skin became more difficult to penetrate with the hypodermic needle, and the collagen content was doubled but still subnormal (113).

Baker, Ingle, Li, and Evans (5) found that the administration of ACTH to rats

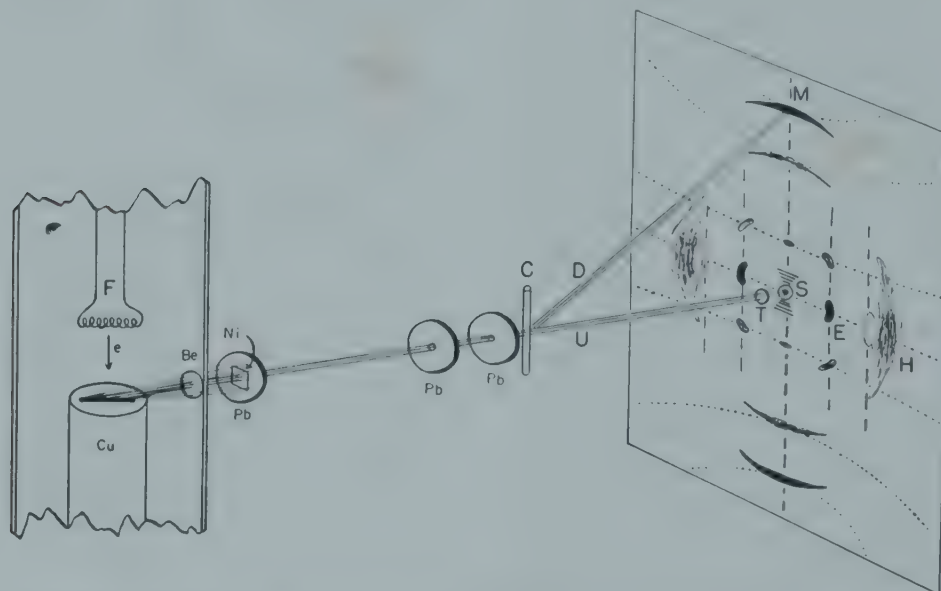


FIG. 4.—Illustrating the X-ray diffraction method as applied to collagenous fibers. Electrons, e , emitted by an incandescent filament, F , are driven by a potential of about 40,000 volts onto the target of copper, Cu , which yields the X-radiation that emerges from the X-ray tube through a window of beryllium, Be . A thin foil of nickel, Ni , transmits principally the characteristic $Cu K\alpha$ radiation (wave length $\lambda = 1.54 \text{ \AA}$) to the collimating system, which consists of fine pinholes cut in lead disks, Pb . Beyond the collagen fiber at C emerge the undiffracted beam U and various diffracted or deviated beams represented by the one marked D . The relatively intense central beam is attenuated by the beam trap, T , whose shadow is seen overlying the center of each of the diffraction patterns in Figs. 5 and 6. The final diffraction pattern, as registered on a photographic film, consists of layer lines (vertical dashed lines) and row lines (horizontal dotted lines) of spots. Prominent features of the collagen wide-angle pattern are indicated by M , the important 2.86 \AA arc on the meridian; E , the $10\text{--}12 \text{ \AA}$ equatorial spot; and H , the diffuse "half-halo" (4.6 \AA). The small-angle layer lines at S generally require finer pinholes and longer collimators and specimen-to-film distances to permit their resolution. In some cases collimating apertures are horizontal slits, which diffuse the diffraction spots along layer lines but retain layer-line sharpness vertically and permit more rapid registration of patterns. From Bear (10). (Reproduced by permission of the author and Academic Press, Inc.)

time, no thinning resulted. In capons testosterone propionate produced an increase in the number of collagen fibers in the skin, whereas in the comb the only change found was a separation and splitting of the collagen fibers produced by apparent imbibition of water by the mucoid ground substance (118). Thyroidectomy in the rat at birth impairs the total skin development (113), the infantile soft short hair and thin skin persisting a long time. The collagen content of

caused the dermal connective tissue to assume a more compact arrangement. When cortisone or compound F was applied locally to the skin in rats, Castor and Baker (24) noted that the dermis became thinner. If the applications were prolonged to 112 days, the collagenous fibers lost their sharp boundaries and appeared to fuse together. The elastic fibers were not affected. Baxter, Schiller, Whiteside, and Straith (7) noted atrophic changes in the epidermis, collagen

fibers, hair follicles, and skin fat in rabbits given cortisone in doses comparable to those used in human patients.

H. STRUCTURE

Earlier schemes were based on wide-angle X-ray spectrography, which, like large apertures of high-power objectives in the ordinary microscope, can be used for resolution of small details of 20 Å or less. With narrow-angle spectrography, which was introduced more recently, comparable to the

its length. Unstretched dried gelatin shows only an amorphous X-ray pattern; but, if stretched, the pattern is practically identical with that of collagen (27).

Besides the wide-angle (small-space) diffraction patterns in collagen, which are indicators of small repeating periods in the molecule (Fig. 5), several investigators using longer X-ray wave lengths and greater distances between specimen and film have found evidence of large-space repeating periods (Fig. 6). In dry collagen fibers periods

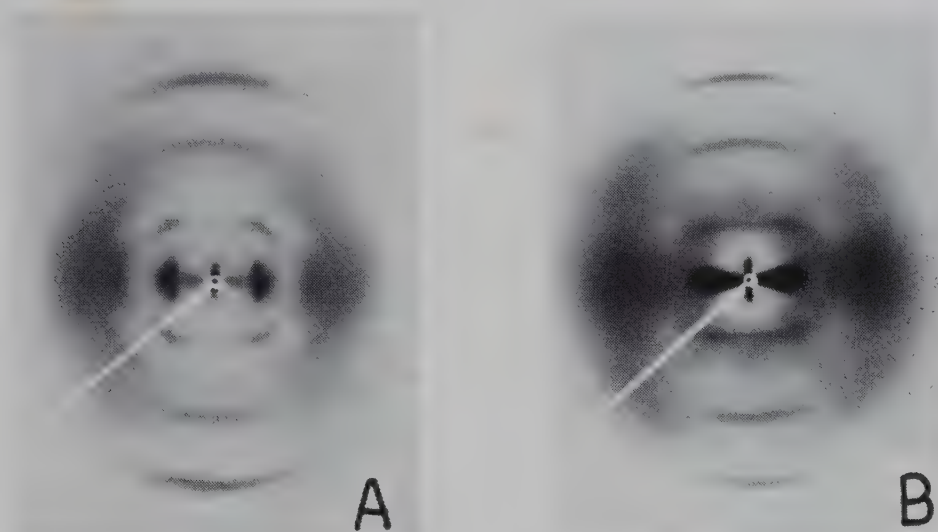


FIG. 5.—Wide-angle diffraction patterns of collagens. *A*, dry kangaroo tail tendon; *B*, moist kangaroo tail tendon. From Bear (10). (Reproduced by permission of the author and Academic Press, Inc.)

small apertures used in low-power microscopy, one obtains resolution of sizes from 20 to 1,000 Å, a range which overlaps that of the electron microscope and can be used for study of fibrillary and nonfibrillary structures (10) (Fig. 4).

Figure 3 is a diagrammatic representation of the wide-angle X-ray pattern of collagen. The so-called “K3 bands,” measuring 2.85 Å, represent an interplanar distance along the fiber axis. Astbury (3) interpreted this distance to be that of one amino acid residue. In silk fibroin, where the polypeptide chains are in a fully extended condition, the corresponding interplanar distance is 3.5 Å. This would mean that the collagen molecule with an interplanar distance of only 2.85 Å must show constrictions somewhere along

of 640–645 Å were demonstrated with this technic (8). In the moistened fiber this period increased slightly, measuring from 665 to 680 Å. These large periods were lost when the collagen fibers were shrunk by gentle heating in water or when gelatin was stretched. The short periods persisted. Altogether, the large periods proved to be less stable than the short ones. No comparable large periods have been demonstrated transverse to the collagen fiber.

The absence of well-defined lateral spacings suggested that the protofibril of collagen is very thin, possessing the width of a very few polypeptide chains, differing in this respect from keratin (8).

Herzog (52, 53) was the first to study periodicities in the structure of collagen by

X-ray diffraction. The first rather complete picture of the structure of collagen was proposed by Astbury (3, 4), who combined data on chemical composition with data derived from wide-angle X-ray diffraction studies. In his scheme periodically repeated groups of amino acids are held to occur in chains of

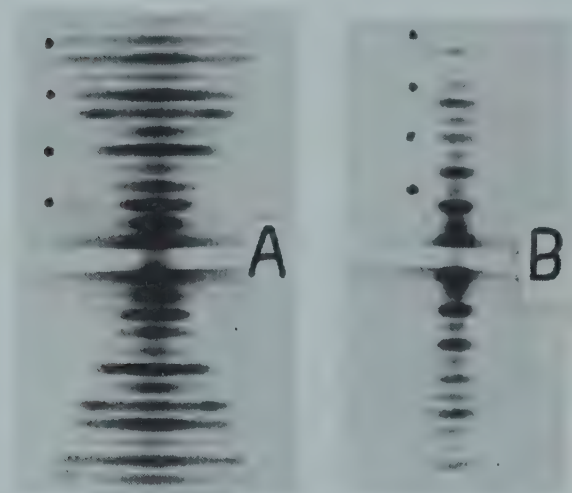
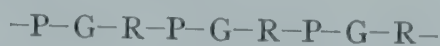


FIG. 6.—Small-angle diffraction patterns of collagens. *A*, dry kangaroo tail tendon; *B*, moist kangaroo tail tendon. From Bear (10). (Reproduced by permission of the author and Academic Press, Inc.)

three, where one-third of the residues are glycine, almost another third are proline or hydroxyproline, and the remaining part are other amino acids. This structure was represented by the symbols



and is more completely illustrated in Figure 7. This arrangement was in line with Bergmann's periodicity theory (p. 340), which has since been found not to be valid for proteins in general but is still thought by some authors to be valid in the case of collagen (70, 68, 69).

In Astbury's model, moderately folded linear main polypeptide chains are lying parallel in sheets which are stacked on one another. He assumed that the imino residues of proline and hydroxyproline are all on one side of the backbone, together with the glycine residues, while the remaining residues are on the other side of the backbone

(Fig. 7, *b*). He held that some spacings in the X-ray diagram of collagen indicate "side chain" and "backbone" spacings, as in keratin, so that elementary fibrils have two dimensions transverse to the fibril axes (3, 4).

Huggins (63) criticized the model of Astbury mainly on the basis that the forces on opposite sides of the chains are unbalanced and that these forces would prevent the main chains from remaining in a straight line and in the same plane; they would bend continuously in the same direction. Instead, Huggins offered the model of a coiled chain with a screw axis (see Fig. 7, *c*). These chains are placed side by side in a thick

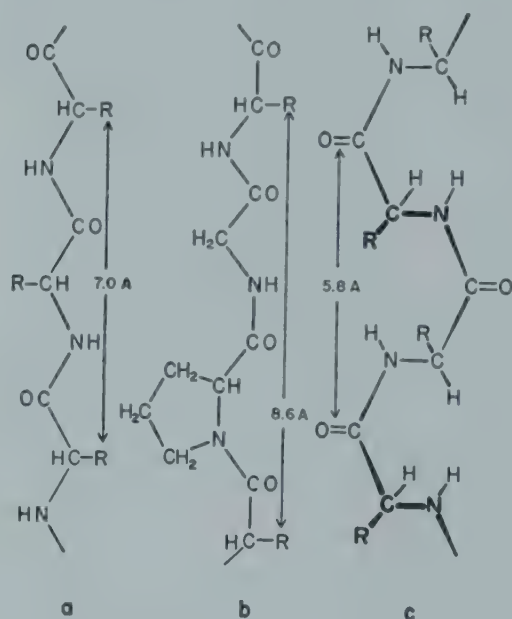


FIG. 7.—Polypeptide chain configuration. Models of (a) Pauling-Corey, (b) Astbury, (c) Huggins (see text). From Bear (10). (Reproduced by permission of the author and Academic Press, Inc.)

plane, joined by hydrogen bonds of the character $>N-H \cdots O=C<$. This model has been objected to by Bear (10) on the basis of some X-ray diffraction findings.

Pauling and Corey (105), proposing new types of structure for proteins in general, developed a special model for collagen, involving an intertwined coil of three chains spiraling around the same axis and connected by hydrogen bonds (Fig. 7, *a*). The

validity of this model has been supported by infrared analysis (2, 105).

As pointed out by Bear (10), all these models were based on the assumption that collagen is not extensible. The connections between the main chains were taken as holding the constituents of the chain rigidly, not permitting any expansion along the main axis. Actually, massive fibers of collagen are capable of being extended only a little beyond the normal relaxed length before rupture occurs. Erroneously, this fact was taken as an indication that the thinnest elements, too—the single amino acid chains—are not at all, or are hardly, extensible. Electron microscope observations of Schmitt, however, left no doubt that the thinnest elements (“protofibrils”) are extensible to quite a considerable degree (p. 407). One of Schmitt’s pictures indicates a ninefold increase in length of a protofibril (Fig. 8). Apparently, massive fibrils, if stretched, may break by collapse of interfibrillary bonding long before sufficient tension has been exerted on individual fibrils.

The most recent and most satisfactory model for the structure of collagen is that of Bear (9, 10). This model takes into account the results of electron microscopic studies as well as those of wide- and narrow-angle X-ray diffraction data.

In Bear’s model, collagen fibrils are composed of thin fundamental units, the protofibrils (Fig. 9). They are normally single, regularly coiled polypeptide chains with about 4.20–5.25 amino acid residues per turn, stiffened by interturn hydrogen bonds arising from the imino and carbonyl groups of the polypeptide main chain. Protofibrils are parallel and arranged axially to one another so that “corresponding particulars of chemical structure more or less match transverse to the fibril axis.” Along the fiber axis there is a long spacing of 640 Å, which indicates a periodically repeated chemical structure of this length. This periodicity corresponds with the cross-striations recognizable by the electron microscope (p. 407). Bear calls the cross-striae “bands,” and the stretches in between, “interbands.” The

chemical configurations of the “interbands” in neighboring protofibrils match perfectly well, while at the level of cross-bands long polar side chains distort the vertical main-chain spirals from their regular course (Fig. 10).



FIG. 8.—Fibril from rat-tail tendon enormously stretched by peeling back of collodion supporting film. From original of Fig. 9, F. O. Schmitt, C. E. Hall, and M. A. Jakus, *J. Cell. & Comp. Physiol.*, 20:21.

The 640 Å periodicity is probably due to interruption at regular intervals of covalent bands. Thus, although the length of the protofibril is indefinite, the length of a single molecule within the protofibril is 640 Å. This molecule contains, according to Bear, 670–700 residues, and their total molecular weight is calculated to be 63,000–65,000. While in earlier models 2.86 Å was taken as the length of a single residue in collagen, Bear’s model postulates that one residue oc-

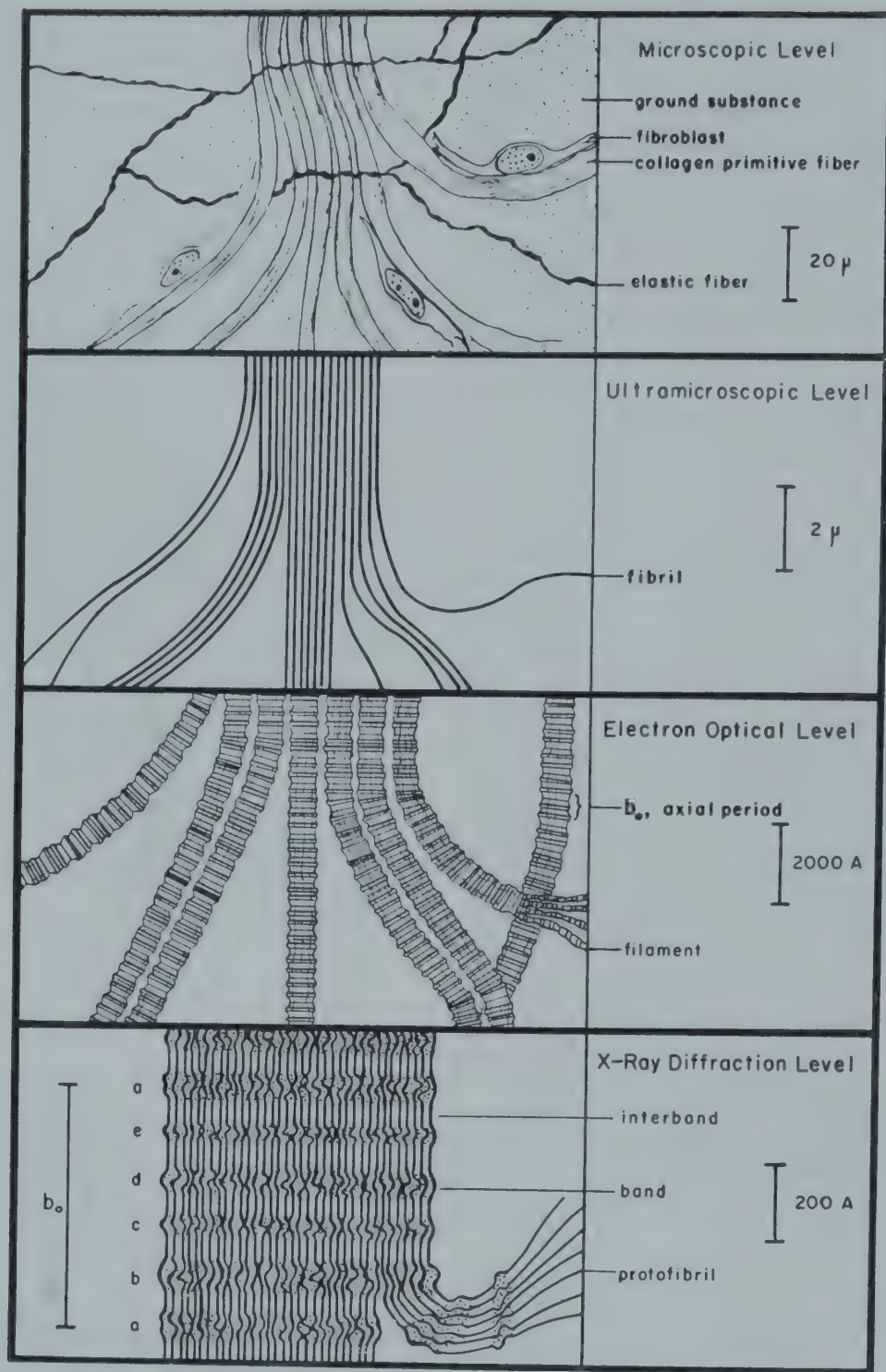


FIG. 9.—Illustrating the various hierarchies of structural elements of collagen. From Bear (10). (Reproduced by permission of the author and Academic Press, Inc.)

cupies only one-third of this length, namely, 0.91 Å. If there are 700 residues, each occupying 0.91 Å length, 700 residues will occupy 637 Å, a figure corresponding well with the measured main periodicity of 640 Å. The interbands between cross-striae contain the average-sized hydroxyl-containing side chains, such as possibly hydroxyproline and

I. BIREFRINGENCE

Amorphous substances whose refractive indices are the same in all directions are described as optically isotropic. All crystals except cubic crystals are optically anisotropic; that is, their refractive index varies with the plane of vibration of the incident light. Birefringence of protein fibers has two

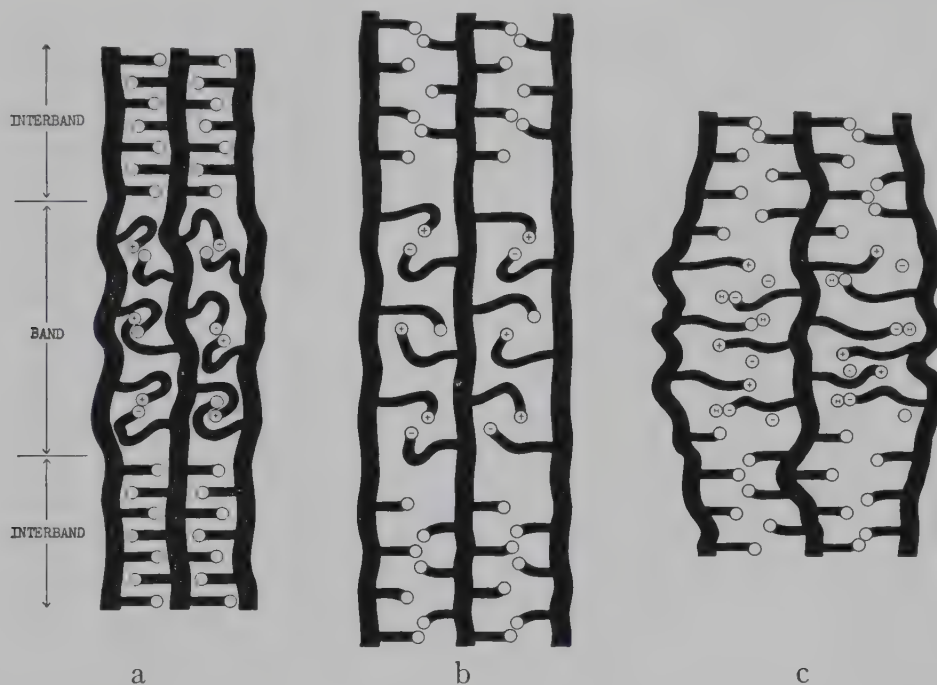


FIG. 10.—Diagrammatic representation of the difference between (a) a dry fibril; (b) a fibril swelling in water at neutrality; and (c) the result of acid swelling. Only polar side chains are shown, with open-circled heads representing uncharged side chains, + and — signs designating correspondingly charged heads or ions, and *H* indicating hydrogen ions. The long charged side chains at bands normally distort the vertical main-chain helices from a straight course. Neutral water (not shown) penetrates bands and interbands, separating main chains to an extent limited by hydrogen bonds between polar heads at interbands; and simultaneously more room becomes available for the charged side chains at bands, which now permit straightening of the main chains. Addition of acid discharges the negative side chains by means of hydrogen ions, and the equal number of free negative ions required to remain at the bands produce local osmotic swellings, which contract the structure axially. From Bear (10). (Reproduced by permission of the author and Academic Press, Inc.)

serine. Nonpolar side chains (glycine, proline, alanine) are distributed through both cross-striae and “interbands.” At the level of cross-striae, salt-type linkages prevail, with positively and negatively charged residues. Swelling in solutions of acids and bases takes place largely at the level of the cross-bands and causes a shortening of the fibrillae (p. 405). Similarly, penetration by stains, such as phosphotungstic acid used in electron microscopic technic, is held to occur at the cross-striae (9, 10).

components: (1) the intrinsic birefringence of the fiber as a whole, analogous to the birefringence of noncubic crystals, and (2) the structural birefringence caused by the composite nature of the fiber. Birefringence of chain polymers, like collagen, provides a measure of the degree of orientation of long molecules parallel to a particular direction. When birefringence diminishes, it means that the parallel orientation of the protein chains of the fibrils is disturbed. As shown by examination in polarized light, collagen

fibrils are strongly birefringent in the direction of the fiber axis, indicating a structure composed of parallel intrafibrillar primary valence chains (92). Alternating light and dark bands have been noted in fresh collagen fibers when viewed under polarized light (66), indicating the composite nature of the fiber.

Formalin fixation does not alter the pattern of birefringence in collagen, while other reagents, such as phenolic compounds, reverse the positive double refraction to negative (10).

J. CROSS-LINKAGES

We have seen that the fibrous proteins are made up of elongated chains of amino acids lying parallel to one another. Collagen, like keratin, has certain cross-linkages present which act as stabilizing forces and prevent the collapse or contraction of the parallel chains. Most important are the hydrogen bonds and salt linkages (p. 357).

The polar side chains forming the salt linkages are rather long and account for the relatively "open structure" of the fiber and its much greater hydration than that of keratin. Sixty-six per cent of the weight of natural collagen is water (75). Because of its relatively large "pores," collagen is penetrated at 20° C. by methyl, ethyl, *n*-propyl, and isobutyl alcohols. The average "pore" size was calculated to be about 7–8 Å, which is greater than in silk or keratin (75). Possibly it is because of this more "open" structure that collagen is more readily attacked by chemical and enzymatic agents than keratin is.

Lloyd and Garrod (75, 72) believe that a special kind of hydrogen bond—the so-called "amide hydroxyl link"—is particularly frequent in collagen:



They held this linkage to be more common than the usual hydrogen bonding between carbonyl and imino groups:



Lloyd (74) has suggested that collagen has two salt links and one amide hydroxyl link to every ten residues. From spatial considerations, however, Weir and Carter (124) believe that salt links may be scarce and that hydrogen bonds outnumber salt bonds by a factor of 7. The disulfide linkage of keratin is apparently absent in collagen (75).

K. GELATIN FORMATION AND SHRINKAGE OF COLLAGEN

The prolonged action of hot water at 80°–90° C. slowly converts collagen to gelatin, i.e., a fibrillary material is transformed into a grossly amorphous mass. The conversion time is shortened at higher temperatures and under pressure (16). The amorphous gelatin, in contrast to the fibrous collagen, is easily digested by trypsin. There must be some change, therefore, in the structure and linkages involved. It is not clear how much hydrolytic splitting is involved in this process. That some disintegration has taken place is indicated by molecular-weight measurements: a mixture of molecules of different sizes has been found in gelatin (64). According to Huggins (62), there is fission of many NHO bridges, followed by formation of bridges in other directions, so that gelatin has an irregular three-dimensional structure, while collagen is more of an assembly of parallel two-dimensional nets. Unstretched gelatin does not give an oriented X-ray pattern, but, if stretched, the small-spacing diffraction pattern of collagen reappears. There is, however, no longer any evidence of the long-spacing pattern (27).

If collagen fibers are *gradually* heated in water up to a temperature of 60°–72° C., the fiber shrinks to about one-third its original length (49). The process probably starts well below this temperature (104, 119). The longitudinal shrinkage of collagen fibers may be involved in the well-known ability of the epidermis to separate from the dermis on a hot plate at 50° C., the loosening being due both to longitudinal shrinkage and to the tendency to go from the gel state of the fiber to the sol state as the temperature is raised, since shrinkage is comparable to a kind of

melting process (25, 85, 35). The shrinkage temperature is a measure of the structural stability of the protein. On heating of collagen, simultaneously with longitudinal shrinkage water penetrates between the grids of the chains, the fiber swells in width, and the side-chain spacing (p. 392) is increased in the X-ray pattern. At extreme shrinkage the X-ray pattern approaches that of unstretched gelatin. If the contracted fiber is moistened and dried under stretch, the wide-angle X-ray pattern again shows perfect fibering but no evidence of the long spacing, indicating breakage of cohesive forces between micelles (27). Shrunk collagen becomes more easily digested by trypsin, also indicating some break in linkages (49).

Lloyd and Garrod held that most probably the salt links were the main linkages holding collagen in the extended condition (74), but Gustavson (49) claims that the main stabilizing forces in collagen are the hydrogen bonds, a majority of these having to be broken before extensive shrinkage of the fiber can occur. Probably both play a role. Shrinkage of collagen fibers is not uniform. With the onset of the process, tiny nodules are formed within the finest submicroscopic fibers, and these increase in diameter as shrinkage increases. The significance of these nodules is not well understood (123, 124). When shrinkage is complete, the collagen fiber becomes rubber-like and can be stretched. If the extension force is released, the fiber returns to its shrunken state (74).

Various substances besides hot water shrink collagen and cause it to pass into a rubber-like state. These are various solutions of salts, their degree of effectiveness following the Hofmeister series (see below). Among cations, Ca^{++} , and among anions, SCN^- are especially effective. Shrinkage is also achieved by treatment with acids at pH's less than 4.0 and by alkalis at pH's more than 10.0. Various organic acids, such as formic, thioglycolic, and lactic, as well as formamide and *m*-cresol, have similar effects (74). Low concentrations of glucose

and sucrose lower the shrinkage temperature and cause considerable swelling of the collagen fiber (75). Formamide, formic acid, lactic acid, thioglycolic acid, and *m*-cresol are all capable of disintegrating the collagen molecule, probably by breaking all hydrogen and salt bonds (75). Dehydrating agents, such as sulfate ions or simple drying of the corium, raise the shrinkage temperature of collagen (71).

L. SWELLING PROPERTIES

An important and distinguishing property of collagen and gelatin is their ability to swell in dilute acids, bases, and neutral salts by combining with these agents (p. 340). A small piece of skin placed in these solutions swells up remarkably. The degree of swelling in acids and alkalis depends not only on the pH but also on the chemical *nature of the acid* or alkali. At the same pH, weak acids have greater swelling properties than strong acids. Divalent bases and acids are less effective (76, 35) than monovalent bases and acids.

In 1888-91 Hofmeister (60) reported on the effects of various anions and cations on the swelling of commercial leaf gelatin and arranged them according to their swelling effect. This series is now known as the "Hofmeister or lyotropic series." Figure 11 shows the degree of swelling of gelatin in various neutral salts at different concentrations. Among anions, the most powerful swelling agents are the thiocyanate, iodide, and bromide ions. The sulfate and acetate ions are dehydrating agents. The effect of monovalent cations on swelling can be listed as $\text{Li} > \text{Na} > \text{K}$ (78). The effect on collagen fibers, although not exactly equivalent, is very similar to that on gelatin. Gelatin swells more readily because the restraining forces of a highly tensile and structured fiber are absent. The mechanism of swelling by neutral salts is referred to as a "lyotropic effect" and is due to a combination of chemical and physical factors (59). Swelling in dilute acids and bases when ionized salts of the protein are formed is, in the main, regulated by the Donnan equilibrium (p. 500).

Neutral salts, such as sodium chloride, generally depress the swelling of gelatin and collagen at acid and alkaline pH values. Salts have a greater depressing effect on acid than on alkaline swelling.

In swollen collagen the shrinkage temperature (p. 404) is decreased. There actually is an inverse relationship between the degree of swelling and the shrinkage temperature. At the extreme acid and alkaline

is first dried, it is more difficult to produce fiber swelling, because, apparently, new cross-linkages are formed in the drying process (73, 77).

Marriott (89) has described in some detail the different processes which may occur in collagen fibers during swelling; among these are (1) the osmotic effect of the Donnan equilibrium, (2) increase in hydration of the protein, and (3) physical separation of

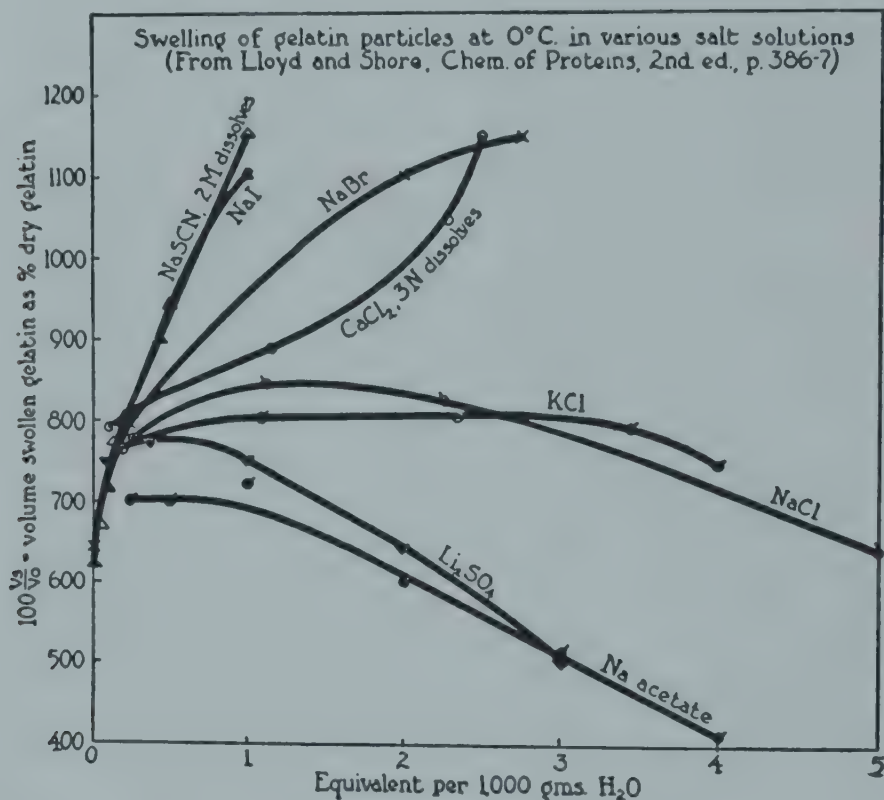


FIG. 11.—Swelling of gelatin particles at 0° C. in various neutral salt solutions. From Felsher (35). (Reproduced by permission of the Williams & Wilkins Company.)

pH values, where swelling occurs at its height, shrinkage of the collagen fiber takes place at room temperature (66). Thiocyanate and iodide ions have a very drastic effect. They shrink and even gelatinize collagen at room temperature (75). Conversely, sulfate, citrate, and acetate ions that dehydrate the collagen fiber increase its shrinkage temperature (75). Swelling of collagen also takes place in some organic solutions, such as thioglycolic acid, lactic acid, formamide, *m*-cresol, and some fatty acids and fatty alcohols (73) (p. 405). If collagen

the fibers and fibrils from one another, resulting in an increase in the total width. In this third process there is no shrinkage in length, such as is seen in true osmotic swelling. Marriott found that separation of fibers of ox hide took place between pH 5.0 and 3.5. True swelling began below this point, with decrease in length of the fiber and further increase in width.

The internal cohesive forces of collagen are able to resist to some degree the distention by swelling agents. In addition, reticular fibers have been demonstrated by Lloyd

(73, 66) to encircle collagen fibers in rat-tail tendon and produce constrictions and bulges in gross fibers as they swell. Day (29) found similar constricting rings in swollen collagen from the deep fascia of rat thigh muscle; but he claimed that these were due to condensation of the "interstitial cement substance" around the fiber. Such condensation occurs below pH 3.5.

If gelatin and collagen are placed in water at or near their isoelectric points, they swell to a slight degree. This is called "imbibition swelling," as contrasted with Donnan swelling. In imbibition swelling, water is held firmly. It requires 8,000 lb/sq in to remove loosely bound water and 30,000 lb/sq in to remove firmly bound water from a gelatin gel at its isoelectric point. Water taken up by acid swelling requires only 1,500 lb/sq in for its removal (78).

M. ISOELECTRIC POINT

Up until a few years ago, it was held that the isoelectric point of collagen, at which swelling ability is at a minimum, was at pH 4.7. This finding, however, was made on collagen which was degraded by alkali when it was prepared. In 1939 both Highberger (56) and Beek and Sookne (13) showed by electrophoretic methods that the isoelectric point of collagen not subject to strong alkaline treatment lies between 7.0 and 7.8.

N. ELECTRON MICROSCOPY

Using a conventional microscope, one finds that the collagen fibers of the skin vary in thickness from about 20 to 40 μ (112). Within the gross fibers, so-called "primitive fibers" can be distinguished, which are about 2 μ in width. These primitive fibers can be further split into fibrils of a uniform width of about 0.5 μ (5,000 A) by teasing with a needle or chemically by electrolytes.

In recent years the electron microscope has thrown further light on the structure of collagen. Yet, in spite of its tremendous power of magnification, this microscope does not resolve the fibers of collagen into their elementary protofibrils.

Under the electron microscope Gross and Schmitt (48) found that the collagen fibrils of human skin ranged in width from 700 to 1,400 A, the majority being 1,000 A wide. In infants some fibrils were found to be as thin as 300 A. The fibrils were straight, unbranched, and imbedded in an amorphous matrix which was not removable by washing with water. The fibrils showed a characteristic cross-striated appearance, which looked the same in all collagens, whether they came from tendon, human skin, or animal skin (Fig. 12).

The cross-striation is due to an alternation of bands of high and low density, and it can be noted that each band extends across the entire width of the fibril. The dark bands have been designated "A" bands, and the light bands "B" bands. Cross-striations can be seen only in dried fibers. No cross-striation is demonstrable in the natural state (38). As mentioned before, in the dried fiber the distance between one dark band and another averages about 644 A (p. 401) (112). After treatment with calcium hydroxide or barium chloride, the striations disappear (100). In dilute acids cross-striation persists, but the interval between the bands is reduced to 540 A. Heat shrinkage apparently does not destroy the striation pattern unless the fiber becomes gelatinized (100, 112).

Individual thin collagen fibrils are markedly extensible. By stretching, the spacing of the A and B bands can be enlarged from the original 640 A to 6,000 A (see Fig. 8, p. 401). Such extensibility is not present in the conglomerate of fibrils as they are represented in coarse collagen fibers. The bundles cannot be stretched because of cohesive forces between fibrils or because of some binding material between them (112). The extensibility of thin fibrils indicates that in the natural state the polypeptide chains of the fibril are in a more folded state than was previously postulated by Astbury and Huggins (3, 63) (p. 400). The type of folding is assumed to be different in the A and B periods (112).

By staining with phosphotungstic acid, Gross and Schmitt (48) found that the A

and B bands further resolved into from three to seven, usually six, intraperiod bands labeled *a*, *b*₁, *b*₂, *c*, *d*, and *e* (Fig. 13). According to Wolpers (126), the dark band normally contains only two intraperiod bands, while the light band has one. When more

points out that Bear's (9) X-ray data on intact tissue support the many-banded intraperiod structure. Pratt and Wyckoff (108) describe a cross-striation pattern somewhat different from that of Wolpers and Gross and Schmitt.



FIG. 12.—Formvar replica of collagen fibrils from adult corium deposited on a glass slide from aqueous suspension and air-dried, shadowed with chromium. 1, fiber bundles adherent to film; in background a few imprints are seen. 2, individual adherent and imbedded fibrils and imprints. From Gross and Schmitt (48). (Reproduced by permission of Dr. Gross and the Rockefeller Institute for Medical Research.)

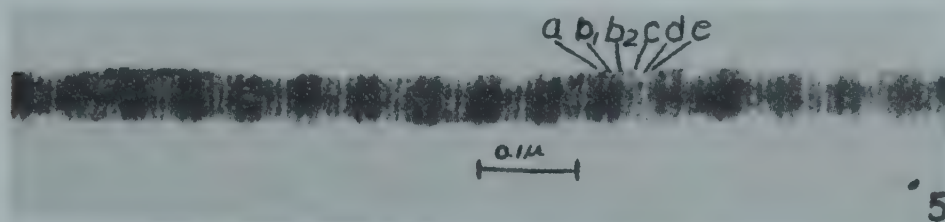


FIG. 13.—Adult corium fibrils from fragmented frozen sections, washed thoroughly in water and deposited on a specimen grid from suspension. Stained with 1.0 per cent phosphotungstic acid. From Gross and Schmitt (48). (Reproduced by permission of Dr. Gross and the Rockefeller Institute for Medical Research.)

bands are found, Wolpers claims that it is an indication of pathological changes in the collagen. He actually produced such changes in the skin of rabbits by anoxemia. Schmitt (111) still maintains that six or seven intraperiod bands are normally present and

In the fibrinoid degeneration of rheumatic nodules and experimental Arthus phenomenon, Wolpers (126) found the normal appearance of collagen under the electron microscope preserved. However, collagen fibers were stuck together abnormally, ow-

ing to fibrin deposition and pathologic changes in the ground substance. There was no swelling of fibers, and the collagen material was apparently intact. Gale (38) likewise found no changes in collagen fibrils under the electron microscope in acute rheumatic fever, rheumatoid arthritis, lupus erythematoses, keloid, fibroma, neurofibroma, and fibrosarcoma. These findings probably justify the criticism of the term "collagen diseases." These diseases are actually diseases primarily of the ground substance (chap. 18).

O. PROCOLLAGEN

Orekhovich *et al.* (102) extracted a "soluble collagen" by the use of a citrate buffer at pH 4 from various tissues, including fresh ox hide, which they called "procollagen." Dialysis of the extracts against water yielded typical fibrils (102). The amino acid com-

position of this material corresponded closely with that of collagen (26). The name "procollagen" would indicate that this material is a precursor of collagen, but there is insufficient evidence for this assumption. Rather, it appears that collagen fibrils can be reversibly solubilized. This behavior is similar to that of epidermal fibrils as described by Rudall (p. 345).

Highberger, Gross, and Schmitt (57) described the electron microscopic appearance of the fibrils formed by the Orekhovich technic. In this material there were typical collagen-type fibrils but also, in addition, fibrils with an axial spacing of about 2,200 Å. It was suggested that the formation of these strange fibrils may be due to the interaction of mucoproteins and soluble collagen, since similar fibrils were obtained by dialysis of solutions of rat-tail tendon collagen in acetic acid with mucoprotein (57) (p. 397).

III. RETICULIN

The reticular fibers of the skin are small in amount, making up about 0.38 per cent of the original dry weight of the collagen (54). These fibers are characterized by their ability to take up silver from silver nitrate solutions. They branch and form a network in the skin. No amino acid analysis of reticulin from the skin has been made. Using fibrous argyrophil tissue prepared from fat and lymph nodes by extraction with concentrated sodium chloride and ethanol, Bowes and Kenten (20), by chromatographic methods, found that, with the exception of a lower proline and hydroxyproline content, the amino acid composition of collagen and reticular tissue was very similar. Probably argyrophil fibers of the skin and other organs are similar to those of lymph nodes and fat tissue (20).

The relationship of collagen to reticulin is not quite clear. Three main theories have arisen concerning this relationship:

1. Reticulin and collagen are entirely different chemical entities. This is concluded from the fact that reticulin takes up silver stain and appears to be more resistant to

acids, alkalies, and boiling water than collagen is (85, 78). In contrast to collagen, it is not dissolved by thioglycolic, formic, and lactic acids at 20° C. and is unaffected by formamide or *m*-cresol at the same temperature (72). Collagen treated with calcium hydroxide becomes susceptible to the action of trypsin, but reticulin does not (65). Reticulin has much less swelling property in acid and base than collagen has (110), although it shrinks in length when the fiber swells and in this respect resembles collagen and differs from elastin (65). Concentrated acids and alkalies do cause swelling and final disintegration of reticulin if boiled (65). It has been claimed that reticulin is not converted to gelatin on boiling (36). McManus (86) found that reticulin stained with periodic acid Schiff's reagent (p. 435), indicating that it probably contains some carbohydrate or that polysaccharide has been adsorbed on, or occluded within, the fine reticulin fibrils.

2. Reticulin is an early developmental stage of collagen. Yuditskaya (127) found that the hide of cattle embryos contained

only one fibrous protein, and this was reticulin. Gross (44) showed that nearly all the fibrous elements of the corium of 2–12-day-old rats were argyrophilic reticulin, with a gradual transition to typical collagen with age.

3. Reticulin is basically collagen. More modern views seem to point to this conclusion (10). Mallory and Parker (87) believed that the only difference between collagen and reticulin was physical in nature, the fine fibrils taking up the silver stain because of their thinness. Histologically, they found evidence that reticular fibers were continuous with collagen. Nageotte and Guyon (95) precipitated fine collagen fibrils from the soluble collagen of rat-tail tendon, which took up the silver stain. Lloyd (72) held that collagen and reticulin were probably similar chemically, except that reticulin seemed more strongly bonded laterally than did

collagen. This could explain the differences in behavior of reticulin toward acids, alkalis, and other chemicals as compared to collagen.

Only little work has been done on the X-ray diffraction pattern of reticulin. Bear (11) found that the wide-angle diffraction patterns of fibers from newborn rat corium, which are argyrophilic, were typical of collagen. Reticular fibers from beef spleen also showed a pattern similar to collagen, with an average repeating period of 655 Å (8). These findings fit in well with those observed under the electron microscope, since the same argyrophilic fibers of newborn rat skin are composed of unbranched fibrils with striations typical of adult collagen (46, 47). These fibrils had the tendency to group in bundles and differed from those of the adult rat by being only one-third to one-half as wide as the latter.

IV. ELASTIN

Knowledge of the chemistry of cutaneous elastin is not nearly so far advanced as is that of collagen. One reason for this is that elastin is not easily available in large amounts in skin, since it makes up only about 2 per cent of the dry weight of human skin. Most of the work on elastin has been done on material from the ligamentum nuchae of animals. We have no definite assurance that these fibers are of the same composition and properties as those in the skin.

Elastic fibers are yellow in color, branched, and of variable width and length (83). In fetuses around the third month, Lynch (81) found a basement membrane in the skin that stained like elastic tissue. This membrane disappeared later. Elastic fibers are strongly acidophilic (83) and are arranged in a superficial subepidermal plexus of fine fibers and a deeper layer of much larger fibers (30). It has been claimed that the elastic fibers play a role in anchoring the epidermis to the corium (91), and a paucity of these fibers has been found in epidermolysis bullosa (33). Histologically, however,

there is no good evidence for this hypothesis (30).

Lowry *et al.* (80) developed a method for the isolation and quantitative determination of elastin from skin by extraction with boiling 0.1 N sodium hydroxide, which destroys collagen and leaves elastin behind, apparently intact. Neuman and Logan (98) have recently separated elastin from collagen by converting the latter into gelatin by hydrolysis with water in the autoclave. This drastic treatment leaves elastin behind as a residue. It is apparent that elastin is more resistant to dilute acids, alkalies, and boiling water than collagen is. Heating elastin in strong acids or alkalies will, however, finally dissolve it (16).

Trypsin definitely has some digestive action on elastic fibers. This can be noted grossly and also under the electron microscope (75, 108, 37) (Fig. 14). Pepsin attacks elastin with difficulty (78). A membrane inclosing the fiber has been found (83, 75). Under the action of trypsin the core of the fiber has been described as first breaking into short cylinders, which later contract

into droplets and remain held in the sheath for some time before the whole fiber disintegrates (75; see also p. 448).

Baló and Banga (6) measured the elastolytic effect of trypsin and chymotrypsin on elastic tissue from the aorta. They found that these enzymes had a much smaller effect than another enzyme which they were able to isolate from pancreatic tissue, called "elastase." The optimum activity of this

results have not been uniformly in agreement. Table 3 shows the amino acid content of elastic tissue as recently compiled by Gross (45). Bowes and Kenten (20) used paper-partition chromatography in the determination of the amino acids of elastin isolated from skin. These chromatograms were not the same as those obtained from the elastin of the ligamentum nuchae, and it may be either that the elastic fibers of the



FIG. 14.—Continuously cross-striated fibrils left behind after tryptic digestion of yellow connective tissue of dog. Palladium shadowing. From A. W. Pratt and R. W. G. Wyckoff, *Biochim. & biophys. acta*, 5:166–74, 1950. (Reproduced by permission of Elsevier Publishing Company, Inc., New York and Amsterdam.)

enzyme occurred at pH 10.3. Action began at pH 6.0. Baló and Banga also found that elastase did not liberate any amino acids from the elastic tissue. The effect of the enzyme was only to transform an insoluble protein into a soluble one. The same was true of the action of trypsin.

The amino acid content of elastin has been determined by several investigators (115, 41, 20, 97), mainly on the material from the ligamentum nuchae of the ox. Various methods have been used in both the purification and the chemical analysis of elastic tissue, and, as is to be expected, the

skin are appreciably different in composition from those of the ligamentum nuchae or that they are less resistant to the boiling water used in their separation and that the residues obtained from the skin contain some other protein breakdown products, perhaps from reticulin (20). From what is known at present concerning the amino acid content of elastin, it appears that this substance contains less arginine, lysine, hydroxyproline, glutamic acid, and aspartic acid, but more valine, than collagen. The basic and acid amino acid content of elastin is relatively low. Stein and Miller (115)

estimated from their determination of the amino acid content that elastin must contain 576 amino acid residues or some whole multiple thereof with a minimum molecular weight of 49,500. Stein and Miller found the isoelectric point by the cataphoretic method to be $\text{pH } 4.18 \pm 0.03$. On the other hand, Bowes and Kenten (20), by the titration curve of elastin with hydrochloric acid in

except by drying. This also can be taken as evidence for the absence of potentialities for forming lateral bonds (74). The few cross-bonds that are present, however, must be strongly held, since elastin is insoluble in all common protein solvents (74). The concept at present is that elastin molecules lie in a randomly crumpled position, the position of maximum entropy, and, if pulled, tend to go back to the latter position (74). The fact that unstretched elastic fibers are not birefringent but begin to show uniaxial birefringence if stretched up to 100–150 per cent of their original length favors this view (92).

X-ray diffraction analysis of unstretched elastic fibers gives only an amorphous pattern characteristic of the unoriented state. The existence of an ordered X-ray diffraction pattern in stretched fibers has not been settled. Kolpak (67) and Astbury (3) found orientation effects in stretched elastic fibers, but it is uncertain whether these were not due to collagen impurities left within the "purified" elastin.

Elastin has much less capability of swelling than collagen has. In pieces of skin swollen by acid treatment, the elastic fibers stand out more clearly as unswollen structures (83). In acids and alkalies some swelling of elastic fibers takes place; but, contrary to what is found in collagen (p. 405), swelling occurs both in the width and in the length of the axis (72, 75). Swelling also occurs in formamide, formic acid, thioglycolic acid, acetic acid, and fused phenol (75).

Electron microscopy of elastic fibers has not as yet yielded much information concerning their structure. Wolpers (125) found the elastic fibers of the ligamentum nuchae to be homogeneous, highly contorted, and branched. Their size varied from 80 to 2,500 Å in width. No cross-striations were seen. Gross (43) at first believed that he saw an ordered structure in elastic fibers partially digested by trypsin. Franchi and De Robertis (37), however, later demonstrated that the fibers which the former investigator saw were not elastic fibers but were probably material from digested bacteria in the unsterile trypsin solutions used.

TABLE 3

AMINO ACID CONTENT OF MAMMALIAN ELASTIN (45)

Amino Acid	Elastin in Cow Lig. nuchae (Gm/100 Gm Protein)
Total N.....	17.1
Amino-N.....	
Glycine.....	29.9
Alanine.....	18.9
Leucine.....	8.7
Isoleucine.....	4.0
Valine.....	17.4
Phenylalanine.....	5.0
Tyrosine.....	1.61
Tryptophane.....	<0.01
Serine.....	0.82
Threonine.....	0.96
Cystine.....	0.15
Methionine.....	0.03
Proline.....	17.0
Hydroxyproline.....	2.0
Lysine.....	0.39
Hydroxylysine.....	
Arginine.....	0.89
Histidine.....	0.07
Aspartic acid.....	0.63
Glutamic acid.....	2.1
Amide-N.....	0.04

the presence of 0.5 N sodium chloride, found the isoelectric point to be more in the neighborhood of pH 6.0.

Elastin differs from collagen in elasticity, the former having rubber-like properties (74, 115) (p. 401). The elasticity has been explained by the fact that the number of polar amino acids in elastin are comparatively few (45). Hence there are few cross-bonds between lateral molecules, and the fibers can be stretched reversibly. Elastin cannot be "set" in the extended position

BIBLIOGRAPHY

1. ALEXANDER, J. Colloid chemistry, Vol. 5, chap. v. New York: Reinhold Publishing Corp., 1944.
2. AMBROSE, E. J., and ELLIOTT, A. Infrared spectra and structure of fibrous proteins, Proc. Roy. Soc. London, s.A, **206**:206-19, 1951.
3. ASTBURY, W. T. The molecular structure of fibers of the collagen group, J. Internat. Soc. Leather Trades Chemists, **24**:69-92, 1940.
4. ———. X-rays and the stoichiometry of the proteins. In: Advances in enzymology, **3**:63-108. New York: Interscience Publishers, Inc., 1943.
5. BAKER, B. L.; INGLE, D. J.; LI, C. H.; and EVANS, H. M. Growth inhibition in the skin induced by parenteral administration of adrenocorticotropin, Anat. Rec., **102**:313-31, 1948.
6. BALÓ, J., and BANGA, I. The elastolytic activity of pancreatic extracts, Biochem. J., **46**:384-87, 1950.
7. BAXTER, H.; SCHILLER, C.; WHITESIDE, J.; and STRAITH, R. E. The influence of cortisone on skin and wound healing in experimental animals, Plast. & Reconst. Surg., **7**:24-31, 1951.
8. BEAR, R. S. X-ray diffraction studies on protein fibers. I. The large fiber axis period of collagen, J. Am. Chem. Soc., **66**:1297-1305, 1944.
9. ———. X-ray diffraction studies. A review of recent researches which concern collagen, J. Am. Leather Chemists A., **46**:438-45, 1951.
10. ———. The structure of collagen fibrils. In: Advances in protein chemistry, **7**:69-160. New York: Academic Press, Inc., 1952.
11. ———. Quoted by GROSS (45).
12. BEEK, J. The carbohydrate in collagen, J. Am. Chem. Soc., **63**:1483, 1941.
13. BEEK, J., JR., and SOOKNE, A. M. Electrophoresis of collagen, J. Am. Leather Chemists A., **34**:641-44, 1939.
14. BERGMANN, M., and STEIN, W. H. A new principle for the determination of amino acids and its application to collagen and gelatin, J. Biol. Chem., **128**:217-32, 1939.
15. BIDWELL, E., and VAN HEYNINGEN, W. E. The biochemistry of the gas gangrene toxins. 5. The kappa toxin (collagenase) of *Cl. welchii*, Biochem. J., **42**:140-51, 1948.
16. BOGUE, R. H. The chemistry and technology of gelatin and glue. New York: McGraw-Hill Book Co., Inc., 1922.
17. BOLDUAN, O. E.; SALO, T. P.; and BEAR, R. S. X-ray diffraction studies of the penetration of stains and tans into collagen fibrils, J. Am. Leather Chemists A., **46**:124-38, 1951.
18. BOWES, J. H. Composition of skin collagen and the effect of alkalis on collagen, Research, **4**:155-62, 1951.
19. BOWES, J. H., and KENTEN, R. H. The amino acid composition and titration curve of collagen, Biochem. J., **43**:358-65, 1948.
20. ———. Some observations on the amino acid distribution of collagen, elastin, and reticular tissue from different sources, *ibid.*, **45**:281-85, 1949.
21. BOWES, J. H., and MOSS, J. A. The free amino groups of collagen, Nature, **168**:514-15, 1952.
22. BRISOU, J., and MILHADE, J. À propos des collagénases, Bull. Soc. chim. biol., **33**:64-68, 1951.
23. CASSEL, J. M., and KANAGY, J. R. Studies on the purification of collagen, J. Am. Leather Chemists A., **44**:424-41, 1949.
24. CASTOR, C. W., and BAKER, B. L. The local action of adrenocortical steroids on epidermis and connective tissue of the skin, Endocrinology, **47**:234-41, 1950.
25. CHERBULIEZ, E.; JEANNERAT, J.; and MEYER, K. H. Über Kollagen und verwandte Substanzen, Ztschr. f. physiol. Chem., **255**:241-54, 1938.
26. CHERNIKOV, M. P. Amino acid content of ox procollagen, Doklady Akad. Nauk S.S.S.R., **67**:345-47, 1949. Quoted by GROSS (45).
27. CLARK, G. L., and SCHAAD, J. A. X-ray diffraction studies of tendon and intestinal wall collagen, Radiology, **27**:339-56, 1936.
28. DANIELLI, J. F.; FELL, H. B.; and KODICEK, E. Enzymes of healing wounds: effect of different degrees of vitamin C deficiency on phosphatase activity in experimental wounds in guinea pigs, Brit. J. Exper. Path., **26**:367-76, 1945.

29. DAY, T. D. The nature and significance of cementing substance in interstitial connective tissue, *J. Path. & Bact.*, **59**:567-73, 1947.
30. DICK, J. C. Observations on the elastic tissue of the skin, *J. Anat.*, **81**:201-11, 1947.
31. EINBINDER, J., and SCHUBERT, M. Binding of mucopolysaccharides and dyes by collagen, *J. Biol. Chem.*, **188**:335-41, 1951.
32. ELSTER, S. K., and LOWRY, E. I. Collagen content of guinea pig tissues, *Proc. Soc. Exper. Biol. & Med.*, **75**:127-29, 1950.
33. ENGMAN, M. F., and MOOK, W. H. A study of some cases of epidermolysis bullosa with remarks upon the congenital absence of elastic tissue, *J. Cutan. Dis.*, **24**:55-67, 1906.
34. FELL, H. B., and DANIELLI, J. F. Enzymes of healing wounds: distribution of alkaline phosphomonoesterase in experimental wounds in rats, *Brit. J. Exper. Path.*, **24**:196-203, 1943.
35. FELSHER, Z. Studies on the adherence of the epidermis to the corium, *J. Invest. Dermat.*, **8**:35-47, 1947.
36. FOOT, N. L. Chemical contrasts between collagenous and reticular tissue, *Am. J. Path.*, **4**:525-44, 1928.
37. FRANCHI, C. M., and DE ROBERTIS, E. Electron-microscopic observations on elastic fibers, *Proc. Soc. Exper. Biol. & Med.*, **76**:515-18, 1951.
38. GALE, J. C. Electron-microscopic studies of collagen from normal and diseased tissues, *Am. J. Path.*, **27**:455-75, 1951.
39. GERSH, I., and CATCHPOLE, H. R. The organization of ground substance and basement membrane and its significance in tissue injury, disease, and growth, *Am. J. Anat.*, **85**:457-507, 1949.
40. GOLD, N. I., and GOULD, B. S. Collagen fiber formation and alkaline phosphatase, *Arch. Biochem.*, **33**:155-64, 1951.
41. GRAHAM, C. E.; WAITKOFF, H. K. W.; and HIER, S. W. The amino acid content of some scleroproteins, *J. Biol. Chem.*, **177**:529-32, 1949.
42. GRASSMANN, W., and SCHLEICH, H. Über den Kohlenhydratgehalt des Kollagens, *Biochem. Ztschr.*, **277**:320-28, 1935.
43. GROSS, J. The structure of elastic tissue as studied with the electron microscope, *J. Exper. Med.*, **89**:699-708, 1949.
44. ———. A study of the aging of collagenous connective tissue of rat skin with the electron microscope, *Am. J. Path.*, **26**:708, 1950.
45. ———. Connective tissue fine structure and some methods for its analysis, *J. Gerontol.*, **5**:343-60, 1950.
46. ———. Aging changes in the collagenous connective tissue of rat skin, *J. Nat. Cancer Inst.*, **10**:1353, 1950.
47. ———. A study of certain connective tissue constituents with the electron microscope, *Ann. New York Acad. Sc.*, **52**:964-70, 1950.
48. GROSS, J., and SCHMITT, F. O. The structure of human skin collagen as studied with the electron microscope, *J. Exper. Med.*, **88**:555-68, 1948.
49. GUSTAVSON, K. H. Evidence for the rupture of intermolecularly coordinated peptide bonds in the heat denaturation (shrinkage) of collagen, *J. Am. Leather Chemists A.*, **41**:47-58, 1946.
50. ———. Action of proteinases on collagen in baiting, *ibid.*, **44**:392-99, 1949.
51. ———. Some contrasting effects of anionic agents on collagens of mammals and fishes, *ibid.*, **45**:789-98, 1950.
52. HERZOG, R. O., and GONELL, H. W. Über Kollagen, *Ber. d. deutsch. chem. Gesellsch.*, **58**:2228, 1925.
53. HERZOG, R. O., and JAHNCKE, W. Über Kollagen. II, *Ber. d. deutsch. chem. Gesellsch.*, **59**:2487, 1926.
54. HIGHBERGER, J. H. The chemistry of collagen. The preparation of collagen from fresh steer hide, *J. Am. Leather Chemists A.*, **31**:93-103, 1936.
55. ———. The basic amino acids of various collagen preparations, *ibid.*, **33**:4-5, 1938.
56. ———. The isoelectric point of collagen, *J. Am. Chem. Soc.*, **61**:2302-3, 1939.
57. HIGHBERGER, J. H.; GROSS, J.; and SCHMITT, F. O. The interaction of mucoprotein with soluble collagen, an electron microscopic study, *Proc. Nat. Acad. Sc.*, **37**:286-91, 1951.
58. HOBSON, R. P. On an enzyme from the blow fly larvae (*Lucilia sericata*) which digests collagen in alkaline solution, *Biochem. J.*, **25**:1458-63, 1931.
59. HÖBER, R. Physical chemistry of cells and tissues. Philadelphia: Blakiston Co., 1945.
60. HOFMEISTER, F. Zur Lehre von der Wirkung der Salze, *Arch. f. exper. Path. u.*

- Pharmakol., **24**:247-60, 1887-88; **25**:1-30, 1888-89; **27**:395-413, 1890; **28**:210-38, 1890-91.
61. HOOKER, C. W., and PFEIFFER, C. A. Effects of sex hormones upon body growth, skin, hair and sebaceous glands in the rat, *Endocrinology*, **32**:69, 1943.
62. HUGGINS, M. L. X-ray studies of the structure of compounds of biochemical interest, *Ann. Rev. Biochem.*, **11**:27-50, 1942.
63. ———. The structure of fibrous proteins, *Chem. Rev.*, **32**:195-218, 1943.
64. KANAGY, J. R. Chemistry of collagen. (National Bureau of Standards Circ. C458.) Washington: Government Printing Office, 1947.
65. KAYE, M. The reticular tissue of the skin, *J. Internat. Soc. Leather Trades Chemists*, **20**:223-29, 1936.
66. KAYE, M., and LLOYD, D. J. A histological and physico-chemical investigation of the swelling of a fibrous tissue, *Proc. Roy. Soc. London, s.B.*, **96**:293-316, 1924.
67. KOLPAK, H. Röntgenstrukturuntersuchungen über elastisches Gewebe unter besonderer Berücksichtigung der Dehnung und Entquellung, *Kolloid Ztschr.*, **73**:129-42, 1935.
68. KRATKY, O. New results on the X-ray patterns of proteins, *Monatsh. Chem.*, **77**:224-50, 1947.
69. ———. Low angle scattering in polymers, *J. Polymer. Sc.*, **3**:195-215, 1948.
70. KRATKY, O., and SEKORA, A. Detection of large distances between lattice planes in kangaroo tail tendons. Molecular structure of fiber proteins, *J. makromol. Chem.*, **1**:113-21, 1943.
71. LENNOX, F. G. Shrinkage of collagen, *Biochim. et biophys. acta*, **3**:170-87, 1949.
72. LLOYD, D. J. Progress in leather science, 1920-1945, chap. iii. London: British Leather Manufacturers Research Association, 1948.
73. LLOYD, D. J.; DEMPSEY, M.; and GARROD, M. The swelling of protein fibers in organic solvents, *Tr. Faraday Soc.*, **42B**:228, 1946.
74. LLOYD, D. J., and GARROD, M. The rubberlike condition of the fibers of animal skin: symposium on fibrous proteins, Society of Dyers and Colourists. London: Chorley & Pickersgill, Ltd., 1946.
75. ———. A contribution to the theory of the structure of protein fibers with special reference to the so-called thermal shrinkage of collagen, *Tr. Faraday Soc.*, **44**:441-51, 1948.
76. LLOYD, D. J., and MARRIOTT, R. H. The swelling of protein fibers. V. The swelling of single collagen fiber bundles, *Tr. Faraday Soc.*, **32**:932-39, 1936.
77. LLOYD, D. J.; MARRIOTT, R. H.; and PLEASS, W. B. The swelling of protein fibers. I. The swelling of collagen, *Tr. Faraday Soc.*, **29**:554-63, 1933.
78. LLOYD, D. J., and SHORE, A. Chemistry of the proteins. London: J. & A. Churchill, Ltd., 1938.
79. LOOFBOUROW, B. S.; GOULD, B. S.; and SIZER, I. W. Studies on the ultraviolet absorption spectra of collagen, *Arch. Biochem.*, **22**:406-11, 1949.
80. LOWRY, O. H.; GILLIGAN, D. R.; and KATERSKY, E. M. The determination of collagen and elastin in tissues with results obtained in various normal tissues from different species, *J. Biol. Chem.*, **139**:795-804, 1941.
81. LYNCH, F. W. Elastic tissue in fetal skin, *Arch. Dermat. & Syph.*, **29**:57-79, 1934.
82. MA, CHUNG K. Morphological and chemical investigation of dermal elastic and collagenic tissue during epidermal carcinogenesis, *Cancer Research*, **9**:481-87, 1949.
83. MACLEOD, J. M. H., and MUENDE, I. Pathology of the skin. New York: Paul B. Hoeber, Inc., 1940.
84. MACFARLANE, R. G., and MACLENNAN, J. D. The toxemia of gas-gangrene, *Lancet*, **2**:328-31, 1945.
85. McLAUGHLIN, G. D., and THEIS, E. R. The chemistry of leather manufacture. New York: Reinhold Publishing Corp., 1945.
86. McMANUS, J. F. A. Carbohydrate specificity of the periodic acid Schiff's reagent, *Am. J. Path.*, **26**:690, 1950.
87. MALLORY, F. B., and PARKER, F., JR. Reticulin, *Am. J. Path.*, **3**:515-25, 1927.
88. MARRIOTT, R. H. The action of trypsin on collagen, *J. Internat. Soc. Leather Trades Chemists*, **16**:6-16, 1932.
89. ———. The swelling of single collagen fiber bundles, *Biochem. J.*, **26**:46-53, 1932.
90. MASCHMANN, E. Über Bakterienproteasen. II, *Biochem. Ztschr.*, **294**:1-33, 1937.

91. MEDAWAR, P. B. Sheets of pure epidermal epithelium from human skin, *Nature*, **148**:783, 1941.
92. MEYER, K. H. Natural and synthetic high polymers. New York: Interscience Publishers, Inc., 1942.
93. ———. Classification of fibrous proteins, *Nature*, **164**:34–36, 1949.
94. NAGEOTTE, J. Sur la solubilité du collagène dans les acides dilués, *Compt. rend. Soc. de biol.*, **98**:15–16, 1928.
95. NAGEOTTE, J., and GUYON, L. Reticulin, *Am. J. Path.*, **6**:631–53, 1930.
96. NEUBERGER, A.; PERRONE, J. C.; and SLACK, H. B. The relative metabolic inertia of tendon collagen in the rat, *Biochem. J.*, **49**:199–204, 1951.
97. NEUMAN, R. E. The amino acid composition of gelatins, collagens, and elastins from different sources, *Arch. Biochem.*, **24**:289–98, 1949.
98. NEUMAN, R. E., and LOGAN, M. A. The determination of collagen and elastin in tissues, *J. Biol. Chem.*, **186**:549–56, 1950.
99. NEUMAN, R. E., and TYTELL, A. A. Action of proteolytic enzymes on collagen, *Proc. Soc. Exper. Biol. & Med.*, **73**:409, 1950.
100. NUTTING, G. C., and BORASKY, R. Electron microscopy of collagen, *J. Am. Leather Chemists' A.*, **43**:96–110, 1948.
101. OAKLEY, C. L.; WARRACK, G. H.; and VAN HEYNINGEN, W. E. The collagenase (kappa toxin) of *Cl. welchii* type A, *J. Path. & Bact.*, **58**:229–35, 1946.
102. OREKHOVICH, V. N.; TUSTANOVSKII, A. A.; OREKHOVICH, K. D.; and PLOTINKOVA, N. E. The procollagen of hide, *Biokhimiya*, **13**:55, 1948; *Chem. Abstr.*, **42**:7805, 1948.
103. PALITZ, L. L., and BRUNNER, M. J. The mucinoses. A classification with histochemical studies on the nature of mucin, *J. Invest. Dermat.*, **14**:159–68, 1950.
104. PANKHURST, K. Incipient shrinkage of collagen and gelatin, *Nature*, **159**:538–39, 1947.
105. PAULING, L., and COREY, R. B. The structure of fibrous proteins of the collagen-gelatin group, *Proc. Nat. Acad. Sc.*, **37**:272–81, 1951.
106. PERRONE, J. C., and SLACK, H. B. The relative metabolic inertia of collagen in the rat, *Biochem. J.*, **48**:iv, 1948.
107. PORTER, K. R., and VANAMEE, P. Observations on the formation of connective tissue fibers, *Proc. Soc. Exper. Biol. & Med.*, **71**:513–16, 1949.
108. PRATT, A. W., and WYCKOFF, R. W. G. The fine structure of connective tissue fibrils, *Biochim. et biophys. acta*, **5**:166–74, 1950.
109. ROBERTSON, W. VAN B.; DUNIHUE, F. W.; and NOVIKOFF, A. B. The metabolism of connective tissue: absence of alkaline phosphatase in collagen fibers during formation, *Brit. J. Exper. Path.*, **31**:545–49, 1950.
110. RODDY, W. T., and O'FLAHERTY, F. O. The behavior of reticular tissue of animal skin, *J. Am. Leather Chemists' A.*, **34**:671–87, 1939.
111. SCHMITT, F. O. Structural and chemical studies on collagen, *J. Am. Leather Chemists' A.*, **46**:538–47, 1951.
112. SCHMITT, F. O.; HALL, C. E.; and JAKUS, M. A. Electron microscopic investigations of the structure of collagen, *J. Cell. & Comp. Physiol.*, **20**:11–33, 1942.
113. SCOW, R. O. Effect of growth hormone on muscle and skin collagen in neonatal thyroidectomized rats, *Endocrinology*, **49**:641–46, 1951.
114. SIZER, I. W. The action of trypsin and other proteases on collagen, *Enzymologica*, **13**:293, 1949.
115. STEIN, W. H., and MILLER, C. G., JR. The composition of elastin, *J. Biol. Chem.*, **125**:599–614, 1938.
116. STOUGHTON, R. B., and LORINCZ, A. L. The action of collagenase on skin and the anti-collagenase factor in human serum, *J. Invest. Dermat.*, **16**:43–52, 1951.
117. STUBBINGS, R. L., and THEIS, E. R. Studies in animal skin proteins. IX. Layer-wise basic and dicarboxylic acid content of animal skin collagen, *J. Soc. Leather Trades Chemists*, **33**:157–62, 1949.
118. SZIRMAI, J. A. The effect of testosterone propionate on the connective tissue of the head appendices and the skin of the capon, *Anat. Rec.*, **105**:337–59, 1949.
119. THEIS, E. R., and STEINHARDT, R. G., JR. Protein axial movement studies. II, *J. Am. Leather Chemists' A.*, **45**:591–610, 1950.
120. THOREAUX. Quoted by GUSTAVSON (50).
121. VANAMEE, P., and PORTER, K. R. Observa-

- tions with the electron microscope on the solvation and reconstitution of collagen, *J. Exper. Med.*, **94**:255-66, 1951.
122. WAKSMAN, B. H., and MASON, H. L. The antigenicity of collagen, *J. Immunol.*, **63**:427-33, 1949.
123. WEIR, C. E. Rate of shrinkage of tendon collagen, *J. Am. Leather Chemists' A.*, **44**:108-40, 1949.
124. WEIR, C. E., and CARTER, J. Rate of shrinkage of tendon collagen: further effects of tannage and liquid environment on the activation constants of shrinkage, *J. Res. Nat. Bur. Standards*, **44**:599-609, 1950.
125. WOLPERS, C. Zur elektronenmikroskopischen Darstellung elastischer Gewebeselemente, *Klin. Wchnschr.*, **23**:169-72, 1944.
126. ———. Elektronenmikroskopische Untersuchungen zur Pathologie der kollagenen Fasern, *Frankfurter Ztschr. f. Path.*, **61**:417-29, 1950.
127. YUDITSKAYA, A. I. The chemical nature of reticulin, *Biokhimiya*, **14**:97-101, 1947; *Chem. Abstr.*, **43**:6263, 1949.

CHAPTER 18

Connective-Tissue Ground Substance

By GEORGE C. WELLS

I. INTRODUCTION	419
A. Physiological Evidence for Presence of Ground Substance	419
B. Constituents of Ground Substance	421
II. THE MUCOPOLYSACCHARIDES OF THE SKIN	422
A. Some Naturally Occurring Mucopolysaccharides	422
B. Hyaluronic Acid	424
C. Chondroitin Sulfate	424
D. The Relationship between Mucopolysaccharides and Proteins of Ground Substance	425
III. HYALURONIDASES	428
A. The Discovery of Hyaluronidase	428
B. Properties of Hyaluronidases	430
C. Hyaluronidase Assay	430
1. Spreading Reaction in the Skin	430
2. Bacterial Decapsulation Method	430
3. Mucin Clot-Prevention Test	430
4. Viscosity-reducing Method	431
5. Turbidimetric Method	431
6. Reductimetric Method	431
D. Mechanism of Degradation of Hyaluronic Acid by Hyaluronidase	431
E. Inhibitors of Hyaluronidase	432
F. Different Actions of Hyaluronidase	432
G. Other Hydrolyzing Enzymes	433
H. Other Spreading Factors	433
IV. HISTOCHEMISTRY AND MICROÖRGANIZATION OF GROUND SUBSTANCE	434
A. Metachromasia	434
B. Stains for Mucopolysaccharides	435
C. Effects of Some Enzymes on Staining Reactions	436
D. Distribution of Mucopolysaccharides in the Skin	437
V. HORMONES AND GROUND SUBSTANCE	438
A. Thyroid Extract and Thyrotropic Hormone	438
B. Sex Hormones	440
C. Cortisone and ACTH	441
VI. CONNECTIVE-TISSUE REPAIR	442
A. The Ground Substance in Wound Healing	442
B. Scurvy	443
C. The Effect of ACTH and Cortisone on Healing	444

VII. THE DEVELOPMENT OF GROUND SUBSTANCE	446
A. Origin of Ground Substance	446
B. Fibrillogenesis and Reconstitution of Fibrils	447
C. Mast Cells	448
VIII. GROUND SUBSTANCE IN SOME PATHOLOGICAL CONDITIONS	449
A. Connective-Tissue Changes in Aging Skin	449
B. Muroid Degeneration and Fibrinoid Degeneration	450
C. Amyloidosis	451
D. Immunity Mechanism and the Connective Tissue	452
E. The Capillary Walls	452
IX. BRIEF REVIEW OF FUNCTIONS OF GROUND SUBSTANCE	453

I. INTRODUCTION

A. PHYSIOLOGICAL EVIDENCE FOR PRESENCE OF GROUND SUBSTANCE

WHEN water or saline is injected into the skin, a wheal is raised, which persists for a considerable time. McClure and Aldrich (207) injected 0.2 ml. of 0.8 per cent saline into the skin of the forearm and found that a raised bleb of about 10 mm. in diameter would persist for about 50 minutes in the normal subject, but for a much less time in patients with cardiac or renal edema. The same is true of loose areolar connective tissue, and the *boule d'œdème* produced by saline injection was noticed long ago by Ranvier. Ordinary microscope sections of these tissues, showing, as they do, wide spaces between the connective-tissue bundles, fail to reveal why water or saline does not disperse immediately through the connective tissue. That there is a tissue fluid between the connective-tissue bundles is usually assumed; but it is necessary to go further and postulate a semigel continuum in order to account for this water binding. Flemming (85), in 1876, brought forward evidence for a ground substance between the bundles of the connective tissue of skin and other tissues and for a cement substance holding together the component fibers of collagen. He observed that acid which caused fiber swelling also induced irregular constrictions along the fibers, suggesting an unyielding effect of the cement substance which was evidently precipitated by acid; there was a *Quellungsan-*

tagonismus between the fiber and its cement, and he thought that the latter was probably a muroid. A vivid picture of the ground substance is conjured up by the work of Bensley (20). She produced a bleb of edema in the subcutaneous tissue of the rabbit by injecting normal saline together with a culture of *Paramecium* adapted to live in it. The whole excised tissue was watched under the microscope, and paramecia were seen to swim freely until halted by an invisible wall at the edges of the saline bleb, where they came up against a barrier of viscous ground substance. She also characterized the ground substance of connective tissue by its different refractive index at the edge of injected saline when the whole specimen was mounted in serum, and by its metachromasia under certain specified conditions in fixed specimens. Partial extractability with saline and with half-saturated lime water suggested a mucin-like component, while the effect of pancreatin pointed to a protein moiety which was easily digested, while connective-tissue fibers were not. Baitzell (9) in the course of microdissection of the living chick embryo found a jelly-like substance between the cells at an early stage of development and suggested that such a milieu is necessary for the support of individual cells if they are to migrate away from original foci of differentiation. Clark and Clark (43) applied to the rabbit's ear the kind of transparent chamber that had previously been used for

microscopy of the living and regenerating tissues in the tails of Amphibia. They noticed that there were no spaces between the regenerating connective-tissue fibers and between the capillaries and lymphatics and that ordinarily there was no Brownian movement or bobbing of cells in the jelly-like ground substance; but if there was edema or exudation, then Brownian movement would be seen. They also found that, in attempting, by micropipette, to inject colloid material such as India ink into the ground substance, considerable force was required but that if the pipette was slightly withdrawn, the ink would flow easily into the "hole" left by the end of the pipette in the gel. McMaster and Parsons (213) injected vital dye into the tip of the ear of the mouse and found that the injection would rupture some lymphatic vessels and allow the dye to ascend intact lymphatics, where it could be watched through a transparent chamber. After a few minutes tiny filaments of dye would project out from the walls of the lymphatics; and when touched with a microprobe, the dye would move and then spring back into place without being dispersed, and only measurably later would touching the dye filament result in its diffusion. This dispersion would be more rapid if the dye were irritating, suggesting that the dye would move away only when "free fluid" was mobilized in the ground substance.

Day (54) was able to show that the delicate subcutaneous fascia of the rat had, besides connective-tissue fibers, an essentially membranous structure. Fine transparent films of fresh tissue were mounted in saline or distilled water under a cover slip and then permeated with dilute acid and examined under high power with dark-ground illumination. At pH 3.6–3.9 he found the refractile connective-tissue fibers to be set in a hazy plane membrane, which itself contained minute fibrillae and had an amorphous opaque background. He likened the appearance to that of "twigs on the surface of a frozen pond." This congealing of the interfibrillary substance was permanent and

could not be altered with dilute alkali or water, and the behavior within a narrow range of pH suggested that of a protein at its isoelectric point. That protein was a major constituent of this membranous substance was also suggested by its rapid disintegration in the presence of trypsin. Further study of similar preparations (55) revealed reversible swelling of the ground substance between pH 4.2 and 11 and under varying conditions of salt environment. Thus at pH 4.3 collagen bundles are tightly packed together, crimped, and opaque, and individual fibrils are not visible under the dark-field microscope. With pH near 11, fiber bundles gradually become swollen and transparent, and individual fibrils in the bundles, while remaining themselves unaltered, become separated and easily visible by virtue of the swelling of the intervening ground substance. At the same time, a shimmering movement of the fibrils is seen; this is a kind of Brownian movement apparent only at certain states of hydration of the tissue; such movement is no longer seen if the ground substance has been removed with trypsin. Beyond the range of pH discussed, i.e., acid to 4.2 and alkaline to 11.1, the collagen fibrils themselves swell, and the whole picture is obscured; and, of course, at the more acid pH, irreversible precipitation of the protein of the ground substance has taken place. In saline a similar hydration of the ground substance occurs and is reversible within the range 2.0–0.125 M. Hydration and swelling of the ground substance take place progressively with increase in sodium chloride concentration. With other neutral salts the capacity to cause swelling is dependent on the ionic strength; and for univalent salts of the same sign the hydration power can be arranged according to the lyotropic series. Day describes the ground substance as a hydrophilic colloid with non-aqueous continuum; its linkages are broken down by trypsin; and it behaves like a protein with isoelectric point between pH 4.2 and 3.8. In the living animal it is suggested that neutral salt is important in governing the state of hydration of the ground sub-

stance. There is much evidence to suggest a polysaccharide component in the ground substance of connective tissues, and its role in tissue permeability is illustrated by further experiments (56). A fine membrane of mouse subcutaneous fascia is attached to the end of a glass tube in a beaker of saline, and the rate of flow of saline through it is measured at a small constant hydrostatic pressure. Addition of hyaluronidase to the perfusate increases the rate of flow, but this effect can be reversed by permeation into the membrane of large-molecule solutions, such as dextran, starch, or agar, after which saline will run through at the original rate. Permeation with trypsin leads to permanent structural damage to the ground substance and to uncontrolled flow of perfusing fluid.

The reversible changes with weak acid and alkali seen in fine subcutaneous fascia are not demonstrable with preparations of rat-tail tendon; for here 90 per cent of the dry weight is collagen and there is very little ground substance. In the teased connective tissue of dermis, reversible swelling of the ground substance between the fibers may be seen under the dark-field microscope in the pH range described by Day. Permeation of saline through the connective tissue of skin is very much slower than through the loose subcutaneous tissue used by Day; but it has been possible (147) to prepare slices of human corium fine enough to allow perfusion but thick enough to avoid the sievelike effect of follicle openings. Mounted between a ground-glass cup junction in a vertical glass tube, this preparation showed acceleration of saline flow with the addition of hyaluronidase and restoration of slower flow after perfusion with starch, which, in turn, could be reversed with diastase. Thus Day's conception of the ground substance (56) of loose connective tissue may be applicable to a denser connective tissue, such as dermis. He considers the interfibrillary ground substance of connective tissue to have an organized network of protein macromolecules, with the interstices of this network filled in with aggregates of molecules of hyaluronic acid.

B. CONSTITUENTS OF GROUND SUBSTANCE

So far we have visualized the ground substance of the connective tissue as semigel/semisol, filling in the spaces between the cells and fibers and vasculature and as a cement between microfibrils in the bundles. In some connective tissues the protein structure of the ground substance is demonstrable, and the mucopolysaccharide component is probably important in water binding and will receive more detailed consideration. The fluid phase of the ground substance is nonetheless an essential component and may be regarded as basically a transudate of plasma carrying metabolic exchange products between parenchyma and capillaries. Dorfman (64) suggests the following constituents for ground substance:

1. Substances derived from the blood stream
 - a) Water
 - b) Inorganic ions
 - c) Glucose, etc.
 - d) Blood proteins
 - e) Other unknown constituents
2. Metabolic products of parenchymal cells
3. Metabolic products of connective tissue
 - a) Fibrous structures (imbedded in the ground substance)
 - (1) Collagen
 - (2) Elastin
 - (3) Reticulin (?)
 - b) Mucopolysaccharides
 - (1) Chondroitin sulfuric acid
 - (2) Hyaluronic acid
 - (3) Mucopolysaccharide protein complexes
 - (4) Others (?)
 - c) Soluble proteins
 - (1) Procollagen
 - (2) Others (?)

While most of this review concerns the more palpable components of ground substance, it must again be emphasized that the physicochemical and staining properties of these gel-like substances are dependent upon their fluid and electrolyte environment. While, normally, free fluid is not discernible, much water is bound in the ground substance, and electrolytes move through "bound" water.

II. THE MUCOPOLYSACCHARIDES OF THE SKIN

A. SOME NATURALLY OCCURRING
MUCOPOLYSACCHARIDES

Mucopolysaccharides of great biological importance are found as essential components of skeletal and other connective tissues in mammals, and they are commonly found in the slimes and gums produced by various bacteria and in bacterial capsular material, where their importance in immunology (126) is considerable. Our knowledge of the chemistry of these substances is limited, and any attempt at classification would be based either on their occurrence in nature or on the products of hydrolysis, in so far as these can be identified chemically. The essential components of the mucopolysaccharides of skin are hexosamine and hexuronic acid, derivatives of either glucose or galactose.

The term "mucin" is used, on the one hand, to describe the naturally occurring slimes and secretions such as the mucus from the goblet cells of the gastrointestinal tract, while, on the other hand, it is used to denote the ropy precipitate which forms when mucopolysaccharide and protein in solution are acidified. The term "mucoid" is even less specific, and one would prefer not to use it, but it is often employed as a general term for the naturally occurring mucopolysaccharides that are found in association with protein. They contain amino sugars and/or aldonic acids. An illustrative classification of these substances is suggested by K. Meyer (178):

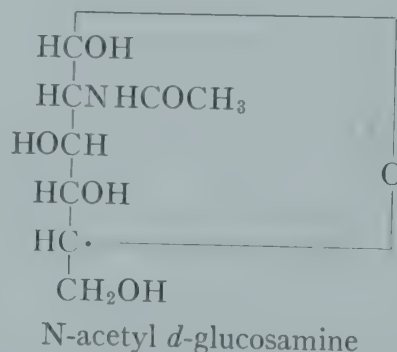
1. Mucopolysaccharides (protein-free), containing amino sugar
 - a) Neutral, containing no acid groups (e.g., chitin, composed of acetylglucosamine only)
 - b) Acid
 - (1) Simple: acid component, uronic acid (e.g., hyaluronic acid, composed of acetylglucosamine and glucuronic acid)
 - (2) Complex: acid component uronic acid and sulfuric acid or phosphoric acid (e.g., corneal mucoid, hog gastric

mucin, heparin, all composed of glucosamine or acetylglucosamine, some uronic acid and sulfuric acid; chondroitin sulfuric acid, composed of acetylgalactosamine, glucuronic acid, and sulfuric acid; certain bacterial acid polysaccharides containing phosphoric acid)

2. Mucoproteins and glycoproteins. These have a relatively small amount of polysaccharide firmly bound to protein. In this group are the serum proteins, ovomucoids, etc.

It may be noted that many substances usually regarded as protein may yet contain small amounts of carbohydrate. For example, highly purified collagen, reprecipitated from citric acid solution, was found to contain about 1 per cent of hexosamine (109); and elastin, after purification and even after reprecipitation following solubilization by elastase (114), still has an important carbohydrate moiety.

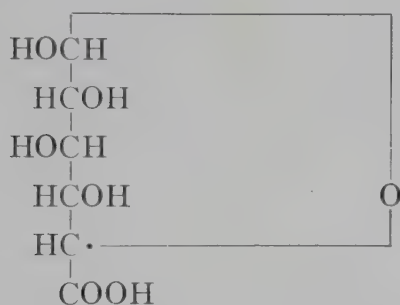
Glucosamine is a stable amino sugar derived from glucose by replacement of a secondary hydroxyl (in the 2-carbon position) by an amino group. 2-Desoxy-2-amino-glucose is the structure of the naturally occurring glucosamine or chitosamine obtained on hydrolysis of certain insect, fungal, and crustacean polysaccharides, as well as many mammalian mucins. In connective tissues, 2-desoxy-2-amino-galactose is present as a constituent of chondrosamine (230). The hexosamine is often acetylated through the amino group:



Glucosamine reduces alkaline copper sulfate. After acetylation it gives a characteris-

tic color reaction with Ehrlich's *p*-dimethylaminobenzaldehyde.

Glucuronic acid is an important component of many natural polysaccharides. It owes its name to its original identification in urine, and it may be noted that many toxins are excreted from the body as uronides. Glucuronic acid is a monobasic acid derived from glucose, with an aldehyde (or hemiacetyl) group remaining at the opposite end of the straight carbon chain from the carboxyl group:



d-Glucuronic acid

The hexuronic acids do not usually reduce alkaline copper sulfate. The naphthoresorcinol test of Tollens is used in the detection of glucuronic acid.

The important linkages between the hexose derivatives in hyaluronic acid and in chondroitin sulfuric acid are glucosidic linkages, but the exact position of the linkages has not been decided, and the chemical structure of these mucopolysaccharides has not been established (190).

A mucoid substance has long been known to exist in the skin, and Van Lier in 1909 (289) extracted "corio-mucoid" from skin, using half-saturated lime water, from which the viscid stringy mucin was precipitated by acetic acid. While this mucin contained protein, it seemed to owe its viscosity to a complex carbohydrate, which, on hydrolysis, yielded some reducing sugar and a sulfuric acid ester similar to the "glucothionic acid" described by Levene (157). In recent years Karl Meyer has pioneered in the work on connective-tissue mucopolysaccharides (179).

Recent developments in the identification

of the connective-tissue mucins date from 1934, when Meyer and Palmer (187) isolated a polysaccharide from bovine vitreous humor. This appeared to be a high-molecular-weight polymer of a disaccharide, consisting of equimolecular N-acetylglucosamine and glucuronic acid; and, because of its origin, they called it "hyaluronic acid." The same mucopolysaccharide was later isolated from human umbilical cord (188), from synovial fluid (191), and from group A and C hemolytic streptococci (144). Hyaluronic acid was also identified in rabbit skin (42), in pig skin (184), in calf skin (189), and in human skin (226).

The acid mucopolysaccharides occur widely in connective tissues as chondroitin sulfates; and Meyer and Rapport (189) have described those that occur in cartilage, umbilical cord, and skin. Treatment of skin with mild alkali, such as half-saturated lime water, while extracting the hyaluronic acid does not extract the chondroitin sulfuric acid. Meyer uses 0.33–0.5 per cent NaOH at 0° C. as the primary step in the removal of mucopolysaccharides from skin; this treatment will split the chondroitin sulfuric acid from its combination with protein without damaging the connective-tissue fibers. After extraction, glycogen must be removed by hydrolysis with amylase. Further steps in precipitation and purification of hyaluronic acid and chondroitin sulfuric acid are described by Meyer and Rapport (189). Meyer and Chaffee (184) took 2.28 kg. of fresh-dissected hog skin, which they extracted with alkali, and the yield of total mucopolysaccharide was 1.85 gm.; and, of this, the sulfate-containing fraction (chondroitin sulfuric acid) was 1.08 gm. Pearce and Watson (226) used a similar method for extraction of the ground substance of human skin which was dissected off eleven amputated legs and pooled. From this material they provided the figures for normal human skin given in Table 1 (p. 424). The analysis here for chondroitin sulfate was incomplete, but those authors have found chondroitin sulfate and hyaluronic acid to be present in human skin in approximately equal amounts.

B. HYALURONIC ACID

Analysis of two samples of hyaluronic acid extracted from pig skin by Meyer and Chaffee (184) provided the data given in Table 2; and, expressed as equivalents per equivalent weight, these amounts approximate unity.

Roseman *et al.* (257) have been able to prepare a highly purified hyaluronic acid

TABLE 1

Water (Per Cent)	Ash (Per Cent)	Hyaluronic Acid (Mg. Per Cent)	Chondroitin Sulfate (Mg. Per Cent)
61	0.7±0.3	24.5±5.7	26.2±4.7

TABLE 2

Nitrogen (Per Cent)	Hexosamine (Per Cent)	Uronic Acid (Per Cent)	Acetyl (Per Cent)
2.99	39.7	41.9	13.1
2.78	37.2	42.0	9.5

TABLE 3

Nitrogen.....	3.72%
Uronic acid.....	10.31%
Carbon.....	44.37%
Hydrogen.....	5.93%
N-acetyl.....	11.74%
Specific optical rotation.....	-67°
Ash.....	Nil
Glycogen.....	Nil

from streptococcal biosynthesis. Their analysis of hyaluronic acid (dried or corrected for moisture) is given in Table 3. This result corresponds closely to theoretical figures based on $(C_{14}H_{21}O_{11}N)_n$.

It is of interest that purified hyaluronic acid is extremely hygroscopic; it is almost impossible to weigh directly such a specimen. It is possible that hyaluronic acid from different sources may show differences in composition, as instanced by rather wide discrepancies in glucuronic acid values.

Stacey (271) gave the approximate values

for hyaluronic acid from various sources as shown in Table 4.

From data on viscosity and streaming birefringence of flow, Blix and Snellman (24) estimated a mean particle length for purified neutral hyaluronate of between 4,700 and 10,000 Å. The highly asymmetric chain is thought to be unbranched. Hyaluronic acid is polydisperse in nature, and its particle size and weight depend upon the source and method of extraction, but a suggested molecular weight was between 200,000 and 500,000, assuming a disaccharide length of 10 Å. That the particles are long is suggested by the great viscosity of watery solutions and by the fact that hyaluronic acid can be

TABLE 4

Nitrogen.....	3-3.5%
<i>d</i> -Glucosamine.....	30-40%
<i>d</i> -Glucuronic acid.....	40-50%
Acetyl.....	8-12%
Specific optical rotation	-60° to -70° in water

spun into "fibers" of considerable tensile strength (179). Hyaluronic acid has no X-ray diffraction pattern and no crystalline structure. Purified hyaluronic acid is not antigenic (144), and hyaluronic acid chemically linked to protein through azobenzyl ether linkages does not act as a specific antigen when injected into rabbits (133).

C. CHONDROITIN SULFATE

Chondroitin sulfuric acid has been found in a wide variety of connective and skeletal tissues. Being firmly bound to protein, it is difficult to extract without markedly altering its physical and chemical properties, and substances of different molecular weights are obtained with different methods of extraction and purification. Chondroitin sulfuric acid, on hydrolysis, yields sulfuric acid, acetic acid, *d*-glucuronic acid, and chondrosamine (2-desoxy-2-amino-*d*-galactose), combined in equimolecular proportions. From measurements of streaming birefringence Blix and Snellman (24) estimated that the mean particle length was about 4,700 Å, with a molecular weight of the order of

260,000. Extraction with calcium chloride and precipitation with hexamino cobaltic chloride yielded a chondroitin sulfate of high purity, which Mathews and Dorfman (172) used for the study of its physicochemical properties. This material gave a molecular weight of 43,300. The chemical analysis of this relatively pure chondroitin sulfate is shown in Table 5.

Meyer and Chaffee (184) extracted chondroitin sulfate from hog skin, and their chemical analysis gave figures for galactosamine, uronic acid, acetyl, sulfur, and nitrogen corresponding with those for chondroitin sulfate from cartilage. Meyer and Rapport (189) have found that chondroitin sulfates from different tissues differ in specific optical rotation, solubility, and resist-

in vitro combination between chondroitin sulfate and collagen, with sulfate as the anion, has been demonstrated (78); but this kind of salt formation occurs only at the isoelectric point of ground substance and will not occur in the physiological range of pH. Bychkov (35) discusses the combination of "procollagen" with chondroitin sulfuric acid, but this also would be unphysiological at pH 4.1. Chondroitin sulfate is very much less viscous than hyaluronic acid and is thought not to play any significant part in water binding. The highly charged anionic groups of chondroitin sulfuric acid probably play an important part in the binding or transfer of electrolytes, or, as Meyer and Rapport suggest (189), it may function as a cation exchange resin.

TABLE 5

Loss on Drying (Per Cent)	Nitrogen (Per Cent)	N-acetyl (Per Cent)	Sulfur as SO ₄ (Per Cent)	Ash as Na ₂ SO ₄ (Per Cent)	—COONa (mEq/ Gm)	Fraction of Theory
6.7	2.9	7.4	6.1	25.7	2.0	0.936

ance to enzyme hydrolysis. Chondroitin sulfate "B" from calf skin and pig skin was found to be distinct from chondroitin sulfate "A" of hyaline cartilage and from chondroitin sulfate "C" of umbilical cord and tendon. Chondroitin sulfate "B" of skin has a specific optical rotation of -50° and is resistant to both testicular and pneumococcal hyaluronidase. It is precipitated from solution in calcium acetate at an ethanol concentration of 20 per cent by volume.

Attempts at purification, while necessary in themselves, may detract from the importance of considering the native state of chondroitin sulfate, which is evidently firmly bound to protein, and its physical properties would depend upon its ionic environment (172). Meyer (182) suggests that the chondroitin sulfuric acid-protein complex contains about 35–40 per cent of carbohydrate. The protein is distinct from collagen and is easily digested by trypsin, with a relatively high yield of tyrosine and tryptophane. An

D. THE RELATIONSHIP BETWEEN MUCOPOLYSACCHARIDES AND PROTEINS OF GROUND SUBSTANCE

While it is clear that chondroitin sulfuric acid in the skin is firmly bound to nonfibrous protein, we have to consider the relation of hyaluronic acid to the protein of ground substance. All workers are agreed that the extraction of hyaluronic acid from skin with alkali degrades the hyaluronic acid and provides no information about its relation to protein. We have then to turn to synovial fluid, where hyaluronic acid can be obtained with less degradation.

Ropes *et al.* (254) were able to compare native synovial fluid with fluid that had been treated with trypsin to destroy the protein and liberate the hyaluronic acid. They found no difference between the viscosity of the joint fluid before and after proteolysis and ascribed the viscosity entirely to hyaluronic acid. Further addition of protein made no change in the viscosity. They found

a constant relation between the log of the viscosity and the square root of the polysaccharide concentration. Synovial mucin (the protein-mucopolysaccharide complex) is base-binding and binds calcium ten times more strongly than does serum protein. It has an appreciable osmotic effect: osmotic effect of serum, 365 mm. water; osmotic effect of synovial mucin, 150 mm. water. If this were on the basis of protein content, the osmotic pressure of the mucin would be 57

in an electrical field has a fast-moving polysaccharide component which is separate from the two slower-moving albumen and globulin components. However, some characteristics of the mucin still suggest a link with protein; for the polysaccharide and protein move as one complex in viscosity and in precipitation and filtration experiments. All these experiments depend upon pH and salt concentration, and there is no doubt that dissociation of hyaluronic acid

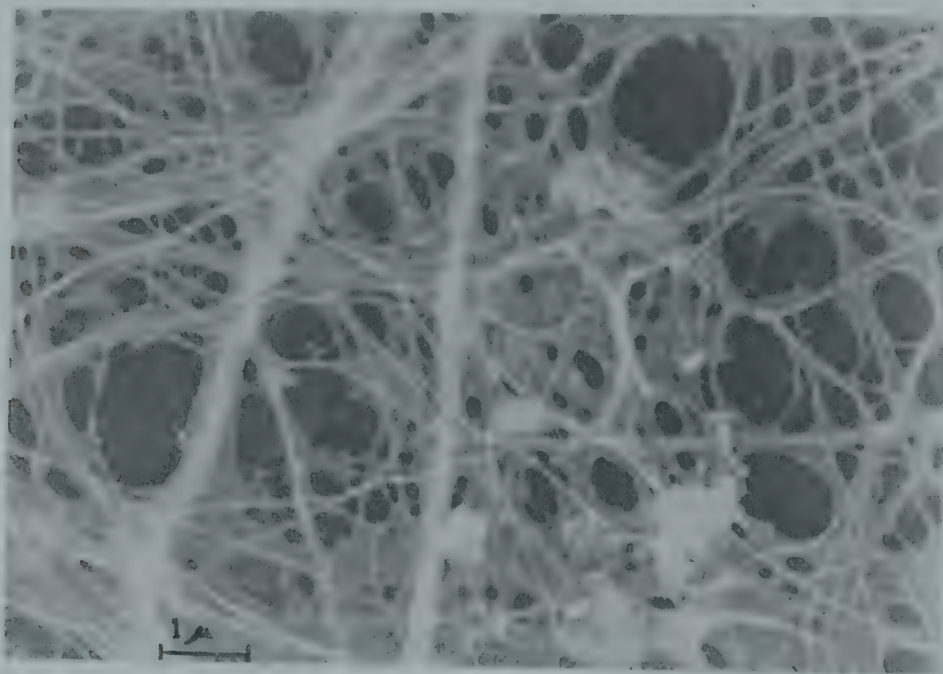


FIG. 1.—Electron microscopic picture of untreated loose subcutaneous fascia from the rat, showing collagen fibrils (with cross-striations just visible) set in an amorphous matrix of ground substance. The electron micrographs were supplied through the kindness of Dr. T. D. Day and Dr. G. Eaves, of the University of Leeds.

mm. water; hence, as the hyaluronic acid is the only other known colloidal substance here, its osmotic effect per gram appears to be about ten times that of albumen.

Meyer (183) considers that hyaluronic acid forms reversible polar complexes with protein but is not chemically bound to it. The sort of mucin complexes produced on the addition of acetic acid are artefacts and do not occur in nature; in fact, at the pH of the tissues, hyaluronic acid may be free of protein. This conception is supported by the electrophoretic studies of Blix (23) and of Ropes *et al.* (254). Synovial fluid at pH 7.6

from protein occurs easily. However, Ogston and Stanier (218) used ultrafiltration as a method of isolating the hyaluronic acid-protein complex from the joint fluid of cattle. They obtained material with higher viscosity than had been obtained by any other means of extraction, and the viscosity was practically the same as that of undegraded joint fluid. They found that the fraction containing the hyaluronate was all within a sharply defined filtration separation and that all such samples contained protein. Any attempt to separate the protein caused degradation of the hyaluronic acid, as

judged by viscosity and ultracentrifugation. The undegraded hyaluronic acid-protein complex consistently contained 7 per cent of nitrogen, of which 5 per cent was glucosamine and 2 per cent was protein. Protein then constituted at least 30 per cent of this mucin complex.

Hyaluronic acid has no regular fiber structure under the electron microscope. Gross (106) obtained highly purified specimens of hyaluronate (umbilical cord) from

dratation at pH 10 in order to allow manipulation as a membrane, after which it was drawn over the grid and dried for electron microscopy. Fine collagen fibrils traverse the picture, and their cross-striations are partly obscured by the amorphous ground substance, which in places has torn into holes on drying. Adequate treatment with hyaluronidase does not remove the amorphous material (Fig. 2); but trypsin removes some of it (Fig. 3). In electron microscopic study

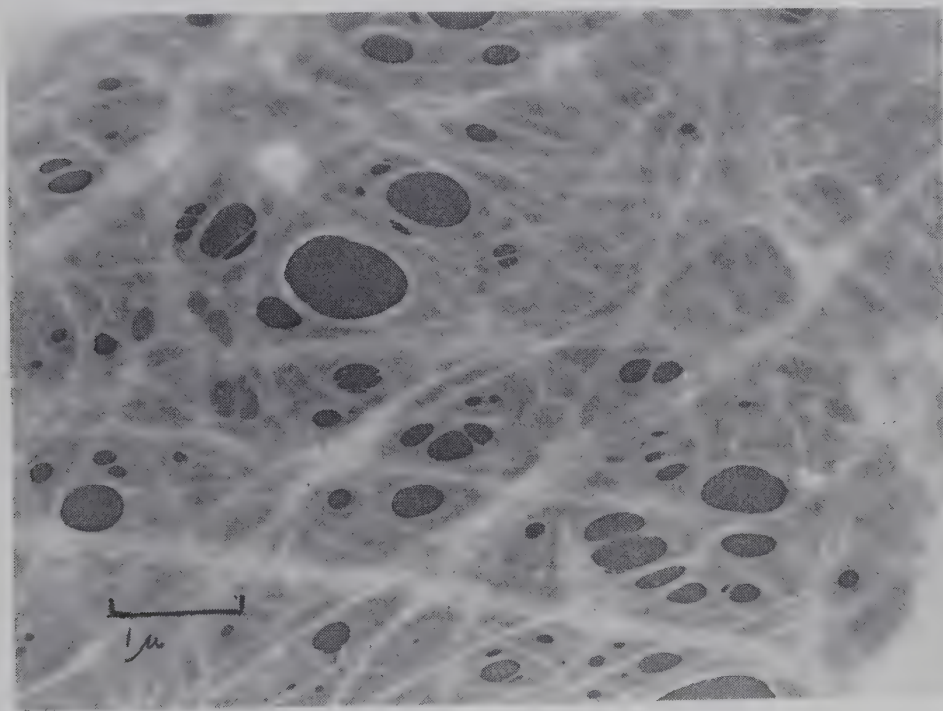


FIG. 2.—The material is from the same source as Fig. 1. The fascia was treated with hyaluronidase. Much ground substance remains unaffected and is seen enveloping the collagen fibrils.

three different laboratories and examined them under the electron microscope. There was unhomogeneous drying of the mucopolysaccharide on the film, giving some membranous appearance, some globular figures, and anastomosing branched fibrous chains; all these were thought to be drying artefacts, depending upon the polysaccharide particle length and degree of depolymerization. There was no cross-striation or regular fiber formation.

Native ground substance appears amorphous under the electron microscope. Figure 1 shows a piece of subcutaneous fascia from the mouse, which was prepared by brief hy-

of skin collagen the amorphous ground substance usually needs to be removed by digestion with trypsin or by manipulation.

Little is known of the chemical synthesis of the mucopolysaccharides. Mosbach and King (198) fed C^{14} -labeled glucose to guinea pigs and found that the carbon chain of glucose is directly available for conversion into glucuronic acid. If, however, C^{14} -labeled CO_2 is fed to animals, it appears first to be converted into glucose or glycogen in the liver before conversion to glucuronic acid.

Dorfman and his associates (257) have nurtured a strain of group A hemolytic streptococcus which gives a good yield of hyal-

uronic acid when cultured on a semisynthetic medium, in which was incorporated glucose labeled with a radioactive carbon. It was found that glucose was the main source of carbon for hyaluronic acid and the 1-carbon labeling was recognizable in the glu-

medium; here, too, the glucuronic acid appears to be a direct derivative of glucose, without rearrangement of its carbon chain.

Nothing is known of the mechanism of polymerization of the mucopolysaccharide complexes, and there is no evidence that

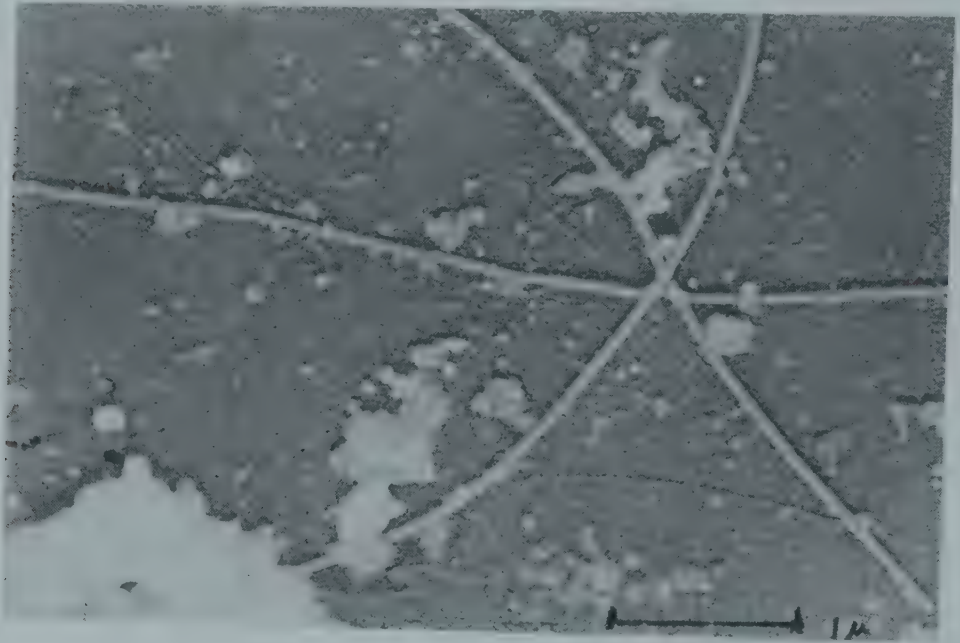


FIG. 3.—The material is from the same source as Fig. 1. Fascial loose connective tissue was fragmented and treated with trypsin. Some amorphous ground substance still clings to the collagen fibrils.

cosamine on hydrolysis, suggesting a direct conversion of glucose to glucosamine without breaking the carbon chain. They also studied (255) the glucuronic acid derived from hyaluronic acid hydrolysis, after inclusion of 6-C¹⁴-labeled glucose in the culture

hyaluronidase plays any part in synthesis (180). There may, however, be enzymatic control of polymerization, as this has been observed in other biological systems to take place even extracellularly (124), either by addition or by condensation.

III. HYALURONIDASES

A. THE DISCOVERY OF HYALURONIDASE

Duran-Reynals (68), in studying the neutralizing effect of immune tissues upon vaccinia virus in animals, discovered that extract of *normal* testis greatly enhanced the virus infection. There was no actual increase in virulence of the infecting organism, and he concluded that there was some effect on the host cells that rendered them susceptible to infection. Extracts of other normal tissues, such as kidney, showed only a very slight enhancing effect on vaccinia, while

spleen extract slightly diminished the infection.

In order to increase the infectivity of vaccinia during animal passage, McClean (200) used an extract of normal testis and got results such as Duran-Reynals had described. In addition, however, McClean noticed that at the site of injection of testicular extract into the skin the wheal would disappear within 30–60 seconds, whereas in the control injection the wheal would persist for at least 30 minutes. He also noticed that

when testis extract together with vaccinia was scarified into the epidermis, there was no enhancement of the virus infection. He deduced that testicular extract increased the permeability of the dermis and in this manner favored the virus, bringing it into contact with more parenchymatous cells. To bring out this point, he injected India ink suspended in saline into the dermis and showed that previous injection of testicular extract, even as long as 24 hours before, would greatly increase the spread of the India ink particles. Spreading factors were discovered in many bacteria (70, 201, 249, 186, 185), in malignant tumors (69, 30, 266), in snake and spider venoms (71, 42), in leeches (46, 47), in animal tissues other than testis (185), and in the cercaria larvae of *Schistosoma* (159).

The mechanism of spreading remained unknown until Chain and Duthie (41) demonstrated the mucolytic activity of purified testicular diffusing factor, using, as substrate, synovial fluid or vitreous humor. There resulted a rapid fall in the viscosity of the mucinous fluid, together with evidence of hydrolysis, in the production of reducing sugar, and in the identification of glucosamine. Heat inhibited this mucolytic effect. They suggested that the action of some spreading factors in the skin could be that of an enzyme breaking down the interfibrillary mucinous substance. The fact that this enzyme hydrolyzed the hyaluronic acid extracts described by Meyer led Chain and Duthie to identify it with the "hyaluronidases" of bacterial origin (186). Robertson *et al.* (249) obtained a mucinase from *Clostridium welchii* cultures which was also capable of hydrolyzing synovial hyaluronic acid.

Chain and Duthie extracted hyaluronic acid from the rabbit skin and showed that it was rendered less viscous by testicular hyaluronidase and that glucosamine was liberated; and the mucin from the sex skin of monkeys responded to hyaluronidase by rapid loss of viscosity. There was, however, no effect upon epithelial mucin (42). They were careful to distinguish from the rapid

hyaluronidase effect a slow spreading effect *in vivo*, where capillary exudate seemed to be an additional important factor; this was produced by a separate component of some bacterial filtrates, venoms, proteolytic enzymes, polypeptides, etc.

While there is no doubt as to the experimental effect of hyaluronidase *on* skin, there is no agreement as to whether it is ever to be found *in* skin. Hyaluronidase has been demonstrated in organs other than the testis (48); and Meyer and his co-workers (185) found appreciable amounts in autolyzed rabbit skin, but none in fresh skin. Meyer (179) thought that the failure to demonstrate hyaluronidase in fresh skin might be due to the presence of inhibitors. R. L. Mayer (176) claims to have found large amounts of hyaluronidase in normal guinea-pig skin, which is increased in skin that has been sensitized and in skin that has been burned. He thinks that there is much hidden hyaluronidase that is released only when the skin is damaged; and he claims that in sensitized inflamed skin the hyaluronidase is increased seventy times above the normal free hyaluronidase. Chain and Duthie (42) were unable to demonstrate hyaluronidase in fresh tissue other than testis; and Glick and his associates (98) could find no hyaluronidase in skin; nor could they (99) demonstrate hyaluronidase in either benign or malignant tumors, provided that they were kept sterile; and they suggest that errors in the past have been due to bacterial contamination of the tissues and extracts. Prose and Baer (239) found no hyaluronidase in human skin, making biopsies from normal and from inflamed skin in allergic conditions. The amounts of skin handled were very small, but the authors were able to recover small amounts of hyaluronidase injected and to demonstrate its presence if the areas of skin were excised within an hour. They were thus of the opinion not only that there was no free hyaluronidase in the material tested but that there was no obvious hyaluronidase inhibitor present in the skin. They were unable to find hyaluronidase in burned guinea-pig skin.

B. PROPERTIES OF HYALURONIDASES

Hyaluronidases are those enzymes which hydrolyze hyaluronic acid or which depolymerize hyaluronic acid without necessarily hydrolyzing to the extent of producing reducing sugars. Meyer and Rapport (190) consider that these two functions depend upon the same mechanism of breaking of glucosidic bonds, though in the case of limited depolymerization the products of hydrolysis may not be detectable.

Hyaluronidases from different sources have different properties to such an extent as to suggest different enzymes or combinations of enzymes. Pneumococcal hyaluronidase produces more complete hydrolysis of hyaluronic acid but fails to digest chondroitin sulfates, differing in both these respects from testicular hyaluronidase (189). Hyaluronidases from different organisms have been shown to be antigenically specific (294).

Purification of hyaluronidase is only relative (190), as electrophoretically homogeneous stable enzymes have not been obtained. A "purified" fresh bovine testicular hyaluronidase (88) in solution was found to be fairly stable between pH 4.0 and 9.5. Total protein was a critical factor in preserving the enzyme, and there was rapid deterioration at less than 1 mg. total protein per milliliter of the solution. Storage at above 0° C. led to a gradual loss of activity. Buffer salts were found to be important in preserving the enzyme in solution and in carrying out dialysis. Exposing the solution to large surface areas, as in shaking and filtration with foaming, resulted in loss of activity. Some hyaluronidases are surprisingly stable on heating and may not be entirely destroyed below 100° C. (190).

For enzyme-substrate reactions, temperatures of 25°–50° C. are used and pH between 4.5 and 6.8. The pH optimum varies with the source of the hyaluronidase and with the salt concentration. The optimum salt concentration varies with the substrate, and the influence of sodium chloride is marked with

turbidimetric and viscosity methods of assay (179). For most reactions 0.1 M sodium chloride is suitable.

Seifter (261) finds that huge doses of purified hyaluronidase injected into animals gave rise to no local or systemic toxic reactions.

C. HYALURONIDASE ASSAY

1. *Spreading Reaction in the Skin*

Colloidal particles of India ink or Evans blue are injected into the skin, together with hyaluronidase, and the extent of spread of the particles is measured in the excised skin when the animal is killed after $\frac{1}{2}$ hour (200, 132). There are many variables that may interfere with the reaction in the living animal (120), such as state of estrus or of hair cycle and whether any inflammation is induced; and the volume of fluid and the pressure of injection are of critical importance. Spreading in human skin has been studied by using as an indicator hemoglobin from rhesus-negative group O blood (130); but this method is objectionable, in that hyaluronidase usually sensitizes and repeat injections are obscured by allergy.

2. *Bacterial Decapsulation Method*

Mucoid colonies of group A and group C hemolytic streptococci have a capsular material rich in hyaluronic acid (203), which can be removed by hyaluronidase. The method is unreliable, in that the streptococci may lose their capsules under other influences than that of hyaluronidase (231), but the method can be applied in the detection of very small amounts of hyaluronidase (199, 93).

3. *Mucin Clot-Prevention Test*

Native hyaluronic acid co-precipitates with protein in the form of a fibrous mucin clot if the solution is acidified. The incubation of the solution with hyaluronidase prevents the mucin clot from forming, and, instead, a turbid suspension forms, and, finally, if hydrolysis has progressed far, the solu-

tion remains clear on acidification (249, 204). This test is applicable only to undegraded hyaluronic acid and will not work with purified substrate.

Other methods depending on altering the physical properties of synovial fluid are the *Spinnbarkeit* method of Gunter (110) and the "ACRA" method of Burnet (33). The latter was found to be sensitive to very small amounts of bacterial hyaluronidase (215).

4. Viscosity-reducing Method

Solutions of hyaluronic acid become depolymerized and disaggregated under the influence of hyaluronidase, and the fall of viscosity may be measured in the Ostwald viscometer (167, 205). Exact regulation of conditions of temperature, pH, salt concentration, and substrate purity are required for reproducible results with an error of about 10 per cent.

5. Turbidimetric Method

Purified hyaluronate at pH 4.2 gives a fairly stable colloid suspension with dilute serum; but hyaluronidase, in depolymerizing the substrate, reduces the turbidity of the suspension, and the turbidity can be measured in the spectrophotometer (143, 65). This method requires relatively little substrate, and large numbers of determinations can be carried out, the error being of the order of 10 per cent. The turbidity-reducing unit (T.R.U.) is that amount of enzyme which in 30 minutes will reduce the turbidity produced by 0.2 mg. hyaluronic acid to the equivalent of the turbidity produced by 0.1 mg. hyaluronic acid.

6. Reductimetric Method

The extent to which reducing sugars are liberated from hyaluronic acid depends upon the hyaluronidase used, the reaction proceeding further with pneumococcal and crude testicular hyaluronidases than with purified testicular hyaluronidase (135, 179). The method is not used for assay of hyaluronidase but is of interest in studying the kinetics of hyaluronidase action (190).

D. MECHANISM OF DEGRADATION OF HYALURONIC ACID BY HYALURONIDASE

Native hyaluronic acid is a high polymer of disaccharide or oligosaccharide units consisting of equimolecular uronic acid and N-acetylglucosamine, of which the glucosamine would give the ordinary reducing reaction with alkaline copper sulfate. Much uncertainty has existed over the mechanism of enzymatic hydrolysis, in view of the difference in effect between purified and crude examples of testicular hyaluronidase (179, 134, 181, 62). Hahn (112) considered that there were two enzymes in testis extract, one a "mucopolysaccharidase," which would break down the long molecules to aldobionic acid groups, and another, "muco-oligosaccharidase," hydrolyzing the aldobionic acid to mono- or disaccharides. Pure testicular hyaluronidase would have only the former enzyme. Pneumococcal hyaluronidase was capable of hydrolyzing pure hyaluronic acid completely and would finish the breakdown to monosaccharide that pure testicular hyaluronidase could not effect (182).

Meyer and Rapport (190) analyzed the products of hydrolysis of hyaluronic acid by adsorbing them in a carbon flow column and then eluting out different fractions and examining them for reducing sugar, N-acetylglucosamine, and glucuronic acid.

With native hyaluronic acid there was a steady reduction in molecular size without liberation of monosaccharides by pure testicular hyaluronidase; and with long incubation the predominating form was tetrasaccharide. On the other hand, crude testicular hyaluronidase would complete the hydrolysis to monosaccharide. A similar step is achieved by β -glucuronidase of liver; this action of crude testicular hyaluronidase is attributed to β -glucuronidase. Purified hyaluronidase seems to attack the large molecule of hyaluronic acid in its center, at first producing depolymerization with marked changes in physical properties, such as change from gel to sol state, loss of power to form mucin clot with protein on acidifica-

tion, and loss of viscosity, while little chemical change can be detected apart from liberation of carboxyl groups. Nonetheless, this part of hydrolysis is attributed to the breaking of glucosaminidic bonds (190). This reaction proceeds with diminishing velocity until all the carbohydrate is in a soluble, diffusible form, such as tetrasaccharide. Breakdown to monosaccharide will occur only if β -glucuronidase is present.

E. INHIBITORS OF HYALURONIDASE

Much work has been done on the inhibitors of hyaluronidase, to which reference will be found in the review of Meyer and Rapport (190). Inhibitors by substrate competition include mucopolysaccharides, such as heparin and chondroitin sulfate. Heavy metals form reversible complexes with the enzyme, notably iron, copper, and zinc salts, though the metal-binding group of the hyaluronidase is not known. Certain polyphenols and quinoids irreversibly inhibit hyaluronidase.

A relatively heat-stable hyaluronidase inhibitor is present in the blood as antibody; this was studied by Friou (91) in connection with streptococcal infections. Serologically distinct hyaluronidases are produced in different tissues and by different organisms and by different strains of streptococci (294). There is also a nonspecific heat-stable hyaluronidase inhibitor present in serum which is probably a polysaccharide (201, 111) and may be heparin (100). Another nonspecific inhibitor, while it may contain polysaccharide, is relatively heat-labile and inhibits hyaluronidases from various sources. It is present in normal serum, but it is increased in various diseases both infective and neoplastic, and its relation to rheumatic fever has engaged particular interest (63, 240). Glick (97) reports very high figures for hyaluronidase nonspecific inhibitor in subacute lupus erythematosus, and some correlation with the sedimentation rate is noted. The system of "invasins" and "anti-invasins" constructed by Haas (111) lacks experimental support.

No consistent relationship has been shown between hyaluronidase production

and the virulence of bacteria (52, 258). The production of hyaluronidase may be adaptive rather than an essential characteristic of the strain of the organism; for Rogers (252) showed stimulation of hyaluronidase production by streptococci and by *Cl. welchii* when hyaluronic acid was added to the culture medium.

F. DIFFERENT ACTIONS OF HYALURONIDASE

Testicular hyaluronidases hydrolyze the chondroitin sulfate of hyaline cartilage and the hyaluronosulfate of cornea (179), but there is apparently no effect upon the chondroitin sulfate "B" of skin (189). Lillie, Emmart, and Laskey (162) suggest that in testicular hyaluronidase there is a chondromucinate separate from hyaluronidase, identifiable by its effect upon chondroitin sulfate, after hyaluronidase has been inhibited. There is, however, not sufficient evidence put forward for identification of a separate enzyme; and, by contrast, Mathews, Roseman, and Dorfman (173) find a close correlation between the effects of testicular enzyme on hyaluronic acid and on chondroitin sulfate, with inhibition running parallel for both activities, and it is thought that the one enzyme—hyaluronidase—hydrolyzes both substrates. Pneumococcal and streptococcal hyaluronidases do not attack the chondroitin sulfates of cartilage and other connective tissues.

Returning to the spreading reaction in skin, it had been apparent that, beyond a certain critical concentration, further addition of hyaluronidase had no effect; Hechter (122, 123) found that further spread depended upon the amount of fluid injected and upon the pressure under which the injection was made, which is in keeping with earlier observations of McClean (200). Chain and Duthie (42) had noted that certain spreading factors, such as snake venom and *Cl. welchii* toxin, produced an inflammatory component which added to the extent of the spread. Hechter explained this in terms of increase of tissue fluid pressure contributing to the spreading effect of the hyaluronidase; and he was able to show that the addition to the hyaluronidase of an agent that promoted

inflammation had an effect similar to that of the toxins and venoms described. This may be an oversimplification, as many snake venoms contain powerful proteolytic enzymes, lecithinases, etc. (236). In this connection Hechter points out that the skin of the living animal is unsuitable for appreciation of the hyaluronidase effect, as the circulatory factor is one of many variables. He advocates the use of freshly excised rabbit skin as a medium for hyaluronidase action, and the test should be read over 5 minutes only. With this method, only hyaluronidase will cause spreading, and factors that appear to inhibit hyaluronidase should be judged by this method. For instance, it has been claimed that cortisone has an antihyaluronidase effect (221) in the living animal; but in excised skin Hechter (123) finds that cortisone does not inhibit the spreading reaction to hyaluronidase, and it seems reasonable to suppose that the *in vivo* effect of cortisone is a suppression of the vascular and exudative components of inflammation, and not directly inhibitory to hyaluronidase.

The effect of hyaluronidase on the hyaluronic acid of skin, allowing rapid spread without inflammation, and the recovery of the ground substance within about 48 hours make it tempting to regard this as a physiological mechanism and to believe that there is normally a balance between enzyme and substrate and perhaps inhibitors in the skin. As there is no clear evidence that hyaluronidase exists in the skin at all, either free or combined and inactive, it is unprofitable to speculate any further in this direction. Similarly, the failure to find hyaluronidase in relation with the joint fluid, either normal or diseased, seems to be evidence against its physiological role here, where the hyaluronic acid itself is so important (243). On the other hand, hyaluronidase is demonstrable in aqueous humor (179), and Meyer considers that it plays a part in keeping the physiological balance between hyaluronic acid formation by the ciliary epithelium and its depolymerization. The hyaluronic acid in the vitreous humor is evidently responsible for its turgor, but the mechanism of its physiological balance is not known.

G. OTHER HYDROLYZING ENZYMES

Collagenase is an enzyme prepared from *Cl. welchii* type A filtrate (170, 216, 21). Macfarlane (208) found that this enzyme would lyse freshly teased human tendon; and Robb-Smith (246) reproduced with muscle *in vitro* certain effects of gas gangrene, such as the destruction of reticulin and collagen support of the muscle parenchyma, leaving the myofibrils intact, though, of course, disrupting the fiber bundles; the effect of collagenase here is the exact opposite of that of trypsin. Unfortunately, this bacterial filtrate contains other enzymes, including lecithinase, hyaluronidase, and perhaps lipase and desoxyribonuclease, and Robb-Smith aptly called it a "bouillabaisse" of enzymes. Gersh and Catchpole (96) and Stoughton and Lorincz (275) found that their collagenase preparations removed periodic acid Schiff (P.A.S.) staining material (p. 435) from sections of skin and, in particular, from the basement membranes, while not primarily attacking collagen. Robb-Smith thought that where *Cl. welchii* invaded the skin and produced bullae at the dermal-epidermal junction, it might be exerting a hyaluronidase effect (246); but this is unlikely, as hyaluronidase normally has no such action. The attack of collagenase on the basement membrane perhaps resembles the action of the pectinases described by McManus (211); but the crude enzymes used here do not permit interpretation of effects in terms of specific substrate structures. It may be noted, however, that polygalacturonidase also appears to attack the basement membranes.

H. OTHER SPREADING FACTORS

Mention should be made of a wide variety of agents that produce spreading in the skin by mechanisms other than mucolysis. These nonspecific spreading factors induce spread of particles only in the skin of the living animal and not in dead skin, as does hyaluronidase. Ascorbic acid and azoproteins (45, 168) have a nonspecific spreading effect and also lower the viscosity of hyaluronic acid *in vitro*. Many spreading factors have no effect on hyaluronic acid, and in this group are

bacterial products, kallikrein, lecithin, and peptones, and simple chemicals, such as glycerol, arsenious oxide, and many others. All these agents tend to produce inflammation and could work by liberation of proteolytic enzymes in tissue, for example. Spector (269) finds that many peptides of approxi-

mate amino acid chain length 8–14 cause increase of capillary permeability and other features of inflammation. Reference may be made to reviews of spreading factors by Meyer and Rapport (190), Meyer (179), Duran-Reynals (72), and McClean and Hale (205).

IV. HISTOCHEMISTRY AND MICROORGANIZATION OF GROUND SUBSTANCE

A. METACHROMASIA

Certain single-color basic dyes, such as methyl violet, thionine, the azures, and toluidine blue, while staining tissues their own color, are capable of forming an entirely different color with certain acidic colloidal complexes. Thus toluidine blue stains most tissues blue (orthochromatic), but connective-tissue mucin is stained pink (metachromatic). This peculiarity of staining in tissue sections is far too complex to allow of interpretation in terms of chemical reaction; but with purified materials in solution, with some control of the ionic environment, some understanding of the phenomenon may be attempted. It concerns the electrical-charge density which may be common to many polyelectrolytes and is not indicative of any specific chemical compound (280). The substances (chromotropes) which produce metachromasia in animal tissue are large-molecule colloids with spaced acidic groups, i.e., $-\text{OSO}_2\text{OH}$, $-\text{COOH}$, and perhaps $-\text{OPO}(\text{OH})_2$, to which the basic dye is fixed. Michaelis and Granich (193) suggested that the dye combines with the chromotrope in such a manner as to form a dimer or polymer of the dye, in which state its color is altered. Levine and Schubert (158) studied the extinction curves for methylene blue by using purified chondroitin sulfate as chromotrope and found little to suggest polymer or dimer formation. The lessening of the metachromasia with higher concentrations of chondroitin sulfate and of neutral salt suggested an evening-out of anionic densities along the molecule; and it is probably the fixing of the dye to areas of high anionic density in molecules of anionic unevenness that alters the light absorption of the dye. The substances ordinarily showing metachromasia are large-

molecule sulfate esters, such as mast-cell heparin, chondroitin sulfuric acid, and mucoitin sulfuric acid. In addition, hyaluronic acid requires special consideration. Meyer (179) says that highly polymerized hyaluronic acid in a concentration of 1 per cent shows metachromasia but that the usual physiological amounts in tissue are not enough to show metachromasia; and he thinks that most naturally occurring metachromasia in connective tissues depends upon chondroitin sulfuric acid. Sylvén (280) goes into this matter in some detail and is in general agreement with Meyer. Sylvén prepared a hyaluronic acid which was free of contaminating sulfuric acid-containing substances (and this is difficult to achieve with hyaluronic acid from umbilical cord, for instance, the sulfate-containing material being probably chondroitin sulfuric acid). The purified hyaluronic acid gave no metachromasia in solution in the sol state up to about 1 per cent concentration. Mixed with gelatin and dried as smears, the hyaluronic acid did give metachromasia if its concentration was as high as 1 or 2 per cent dry weight of the gelatin mixture. Hyaluronic acid was considered to be borderline between metachromatic and nonmetachromatic and is very much weaker in this respect than chondroitin sulfate. Metachromasia, Sylvén concluded, was of little value as a staining method for hyaluronic acid, since the method will not distinguish between different colloidal electrolytes.

Under certain conditions other nonsulfated compounds may show metachromasia. Bignardi (22) has shown that when glycogen is treated with chromic acid beyond the optimum for the Bauer stain (16), so that the Schiff reaction becomes weak or negative,

then the glycogen is found to stain metachromatically. Gomori suggests (105) that the release of many carboxylic groups during the oxidation may give rise to this effect. Indeed, McManus (211) states that any polysaccharide during oxidation may at a certain stage show metachromasia. Furthermore, different methods of fixation produce different results, and a comparison in metachromasia can be made only with standard methods of fixation and of dilution and pH of stain, as was pointed out by Bensley (20) and by many subsequent workers. Follis (87) has shown that unfixed skin, if pretreated with pepsin (but not with trypsin), will have its collagen bundles rendered metachromatic when subsequently stained; whereas, with fixed skin, either of these two enzymes will predispose the collagen bundles to stain metachromatically. One might suggest here that the proteolytic enzyme might first attack the protein complexes of the ground substance, which, in turn, might release acidic groups of acid mucopolysaccharide, such as chondroitin sulfate, which would then give the metachromatic effect. French and Benditt (89) found that histone, albumen, and globulin would block the metachromatic staining of cartilage by basic dyes.

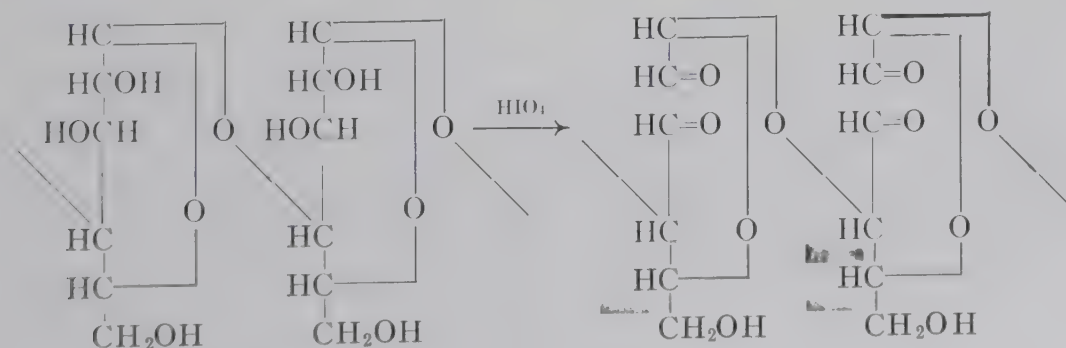
Metachromasia of the ground substance is to be found in embryonic tissues (298), usually in regenerating connective tissue, such as may be found in healing wounds (p. 443), and in the uterine submucosal stroma following menstruation (20). The adult skin has normally no metachromasia, though Wislocki has found a small amount in the papillae (298), and Montagna (194) found metachromasia in relation with growing human hairs, both in the connective tissue of the papillae and in the epithelium of

the external root sheath. The same relationship between metachromasia and actively growing hairs was recorded by Sylvén in the rat. This metachromatic material could be removed with hyaluronidase. Mast cells in the skin consistently show metachromasia by virtue of their content of heparin (monosulfate); this will be discussed on page 448.

B. STAINS FOR MUCOPOLYSACCHARIDES

The chondroitin sulfate of connective tissue has a strong affinity for iron and copper, and staining methods have been based on this property. Bensley (20) mordanted fresh connective tissue with copper sulfate and then stained with colorless haematoxylin and demonstrated a blue homogeneous coating of the connective-tissue fibers. Hale (113) used ferric hydroxide followed by the Prussian blue reaction and showed acid polysaccharide binding of iron. Neutral polysaccharides do not stain by this method. These stains are not very satisfactory and are by no means specific for polysaccharides.

Periodic acid serves to split the carbon chain of a carbohydrate between the first and second carbon atoms if glycol groups are attached at the 1 and 2 positions or if such glycolic groups have hydroxyl groups replaced by amino or alkylamino groups. At the split in the carbon chain, aldehydes are formed. In the case of polysaccharides the reaction takes place without depolymerization, so that the product of oxidation is not broken up or washed away and the aldehyde-containing polysaccharide can then be detected by the formation of a purple color with Schiff's leukobasic fuchsin. This is the basis of the periodic acid Schiff stain (P.A.S. stain) described by McManus (209) and by Hotchkiss independently (131).



The polysaccharide has vicinal $-OH$ groups oxidized to aldehydes with a break in the carbon chain here. There is no depolymerization, owing to the 1C to 4C linkages between two hexose units (105).

McManus considers that P.A.S.-positive staining is usually indicative of 1-2 glycols and their amine substituents and hence is indicative of carbohydrate. Further evidence can be gained by acetylation and then mild saponification. Acetylation blocks the P.A.S. reaction by combining at either glycol or amino or carboxyl groups. If the P.A.S. staining is then restored by brief saponification with mild alkali, this is evidence of glycol groups, because acetyl is not so easily removed from amino radicals (210). According to Wolman (301), some lipids still give a positive reaction.

It is important to have control sections treated with the Schiff reagent without previous periodation, as this will show up some naturally occurring aldehydes, such as ceroid and elastic-tissue aldehyde (104). Certain lipids give a positive P.A.S. reaction; but if these have not been removed during the preparation of the section, they may be removed with hot methanol and chloroform. In order to get comparable results, the details of the conditions of staining and especially the pH must be watched, and Lillie (161) emphasizes the difference between the results obtained by using the McManus procedure and the Hotchkiss method. Glycogen stains intensely with the P.A.S. method and is readily seen in the skin. It is distinguished from other polysaccharides by its removal with amylase or ptyalin. With this method of staining, amyloid material from different sources gives different results, some negative and some positive. Considerable uncertainty exists over the staining of fibrin (57).

The distribution of P.A.S.-positive material in normal human skin was described by Stoughton and Wells (276). While there is some diffuse staining of all the connective tissue, particular condensations are seen at the junctions of connective tissue with epithelium (basement membranes) and out-

side the endothelium of the capillaries. These amorphous membranes are closely associated with the network of finest connective-tissue fibers (reticulum); but silver staining with superimposed P.A.S. staining shows that the reticulum can be resolved from the P.A.S.-positive material (161). Ritter and Oleson (245) used a combination of Hale stain and P.A.S. procedure to emphasize the difference between the staining of the basement membranes and the rest of the connective tissue. Failure of a tissue to stain by any of the methods described seems insufficient evidence for suggesting the absence of mucopolysaccharide (58), because chemically important amounts of carbohydrate may not be detectable by any staining method.

C. EFFECTS OF SOME ENZYMES ON STAINING REACTIONS

The effect of specific enzymes on the characteristic staining of certain substances in tissue or sections may be used in a further attempt to identify these substances (211). For instance, hyaluronidase, in removing metachromatic staining from sections of myxedema cutis, would appear to be hydrolyzing mucopolysaccharide material. But a similar removal of metachromatic material could result from incubation of the section with proteolytic enzymes or with alkaline buffer, so that staining removal must be interpreted with caution as regards specificity. Benditt and French (17) studied the removal of metachromasia from acetone-fixed sections of cartilage in conjunction with changes in the incubating medium. Removal of metachromasia with malt extract resembles the effect of trypsin and of chymotrypsin where tyrosine is liberated in the incubating mixture. Pure hyaluronidase, on the other hand, gave no evidence of proteolysis; and hexosamine and mucins were identified in the incubating fluid. Sections incubated with histone showed loss of metachromasia without evidence of hydrolysis, and here an increase in weight of the treated sections was measured. Histone had become

attached to the acidic groups of mucopolysaccharides, blocking the staining with basic dye. Thus with crude tissue extracts protein may compete with basic dye for the polysaccharide, so that staining loss is due to masking and not to hydrolysis.

We know nothing of the structure of the strongly P.A.S.-positive basement membranes beyond the bare facts of their staining properties. It is assumed that these contain mucopolysaccharide, but so far there is no absolute proof of this. Hyaluronidase has no effect upon basement membranes (276, 161), though, with freeze-dried sections, after many hours' incubation, the removal of P.A.S.-positive material was reported (96). Proteolytic enzymes remove the P.A.S.-positive material fairly rapidly, and an admixture of small amounts of proteolytic enzyme with carbohydrate-hydrolytic enzymes, such as hyaluronidase, might account for discrepancies, just as the malt extract already mentioned (17) was found to be partly proteolytic. Certain samples of collagenase and of *Cl. welchii* filtrate seemed to remove the P.A.S.-staining material from the skin (96, 275), whereas other examples of this enzyme group have preferentially attacked reticulin and collagen (246). McManus (211) claims that pectinases and polygalacturonidase selectively remove the P.A.S.-staining material from acetone-fixed sections; but collagenase and pectinase are crude collections of enzymes (247), and at present no conclusions can be reached concerning the specificity of the staining changes that they induce.

D. DISTRIBUTION OF MUCOPOLYSACCHARIDES IN THE SKIN

The use of freeze-drying in vacuo as a method of preparation allowed Gersh and Catchpole (96) to explore the P.A.S. staining of tissues with confidence that artifacts were for the most part excluded; and their results showed essentially the same P.A.S.-positive areas as were found with conventional fixation. It was considered that strongly P.A.S.-positive areas were indicative of insoluble mucopolysaccharide com-

plexes; and the authors postulated that certain differences in the physical state and staining in these areas might be due to alterations in the state of polymerization of the carbohydrates of the ground substance. In a highly polymerized state the ground substance forms a gel which is not soluble in water; the staining with P.A.S. is light; metachromasia is not present; and the uptake of Evans blue is slight. With partial depolymerization, the ground substance is in a semisol state but is not soluble in water; the P.A.S. staining is very strong; there is strong metachromasia; and Evans blue uptake is strong. With further depolymerization, the ground substance becomes watery and water-soluble; the P.A.S. staining becomes weak; metachromasia is variable, though Evans blue uptake may be strong. This attractive theory (94, 95) has aroused wide interest in the ground substance; but it should be pointed out that simple depolymerization as applied to such a material as hyaluronic acid or chondroitin sulfate would not account for the alterations in staining discussed, as far as the chemistry of these reactions is known. Moreover, some of the phenomena could be accounted for on the basis of changes in hydration in the ground substance or of inorganic ions, or there may be actual hydrolytic degradation of the polysaccharide chains. Another important concept emphasized in this work concerns the connective-tissue changes which may take place in specialized areas. In the pregnant guinea pig the symphysis pubis widens just before parturition, and this widening is the result of changes in the connective tissue in this area, whereby the ground substance becomes abundant, metachromatic, and hydrated—changes described as “depolymerization,” probably mediated by fibroblasts specially responsive in this area to hormone stimulus. Perl and Catchpole (229) were able to bring about similar changes in the pubic connective tissue in virgin guinea pigs by preparing them with estradiol and then giving them serum containing relaxin. The same kind of change in the ground substance toward a water-soluble and more metachro-

matic state is seen in the stroma of organs undergoing cyclic change, such as the ovary or the uterus, the connective-tissue changes being apparently reversible. This degradation of ground substance is also described in relation to wound repair and in the connective tissue bordering malignant tumors.

Apart from the special condensations of ground substance staining strongly with the P.A.S. method, there appears to be a diffuse coating of the connective-tissue fibers which is not very amenable to microscope study. In addition, there is a cement substance between the fibrillae which make up the collagen bundles; but higher magnification is needed to appreciate these, and the best evidence is derived from the work of Day (55) already discussed and from electron microscopy, which reveals that amorphous material must be removed before the collagen fibrillae can be clearly photographed (107).

Where reticulin is increased, there also will be found an excess of amorphous ground substance which may be stained by the

P.A.S. method or metachromatically; but the relation between these two elements is obscure. Reticulin appears to be no different from collagen under the electron microscope as far as the protein fibrils are concerned (p. 410); but it is curious that this fine-branching, silver-staining network does not swell or go into solution with acetic acid, as do the larger collagen fibers (247). The silver impregnation may depend largely upon the diameter of the fibers, though proximity to epithelium may be a factor in silver staining (15).

Evidence of carbohydrate staining may usually be found where elastic fibers are abundant, as in large vessel walls (32), and electron microscope study of elastica shows much amorphous material (302). Examination of elastic fibers dissolved by elastase (12) suggested to Hall *et al.* (114) that the central core of elastic protein was surrounded by a sulfuric acid- and carbohydrate-containing protein complex which they called "elastomucin."

V. HORMONES AND GROUND SUBSTANCE

A. THYROID EXTRACT AND THYROTROPIC HORMONE

The curious nonpitting edema seen in the skin of patients with gross hypothyroidism suggested accumulation of water-binding mucin and hence the name "myxedema" (222).

Byrom (36) measured the fluid, electrolyte, and protein loss in subjects receiving thyroid extract. In the normal subject receiving thyroid there is loss of fluid, in which there is more potassium than sodium, indicating mainly loss of intracellular fluid (p. 500), whereas in myxedematous subjects the fluid loss was accompanied by excess sodium, indicating loss of extracellular fluid. Byrom related the fluid in myxedema to extracellular protein accumulation and suggested that this protein ("mucoprotein") must have a great osmotic effect. The importance of the carbohydrate component of the extracellular mucin in fluid and sodium retention was not at that time appreciated. It

has been clearly recognized that mucin accumulates extracellularly in myxedema and that the tissue rather resembles that of the embryo; thyroid prevents mucin accumulation. This effect of thyroid is not purely one of increase of metabolic rate, for dinitro-orthocresol will increase the metabolic rate without altering the accumulations of ground-substance mucin in myxedema (61).

Brewer (31) reported autopsy findings in advanced myxedema and found gross accumulation of metachromatic material in the skin, which was considered to be acid mucopolysaccharide. Asboe-Hansen (3) deprived hypothyroid patients of thyroid extract in order to make them experimentally myxedematous. In the state of myxedema there was accumulation in the dermis of metachromatic material and of Ehrlich's mast cells. The resumption of thyroid medication resulted in the removal of this metachromatic material and a reduction in mast cells. The intradermal injection of saline in

myxedema results in a discrete accumulation of fluid, which remains at the injection site for an abnormally long time; this effect is reversed by hyaluronidase. Asboe-Hansen considers that the excess of thyroid-stimulating hormone of the anterior pituitary is an important factor in the production of the connective-tissue changes of myxedema; and he suggests that it is the mast cells that are stimulated to produce an excess of hyaluronic acid. At present there appears to be insufficient evidence for ascribing this specific property of hyaluronic acid formation to the mast cells; but this will be discussed on page 448.

Werner (295) treated myxedematous patients with ACTH and found that after 3 days the mucinous infiltration of the skin involuted, the skin becoming warm and

curious paradox, in that the condition appears in patients with hyperthyroidism or after treatment for hyperthyroidism. In these thick plaques on the shins there is edema without pitting; incision shows a gaping firm tissue with little tendency to bleed;

TABLE 6

	Relative Viscosity at 38° C.	Mucin Nitrogen (Gm/100 Ml)
Myxedema joint fluid	361	0.192
Normal human joint fluid . .	208	0.066

and a thick mucus can be squeezed out of the skin. Sections show copious metachromatic material between the connective-tissue bundles in the dermis, which can be re-

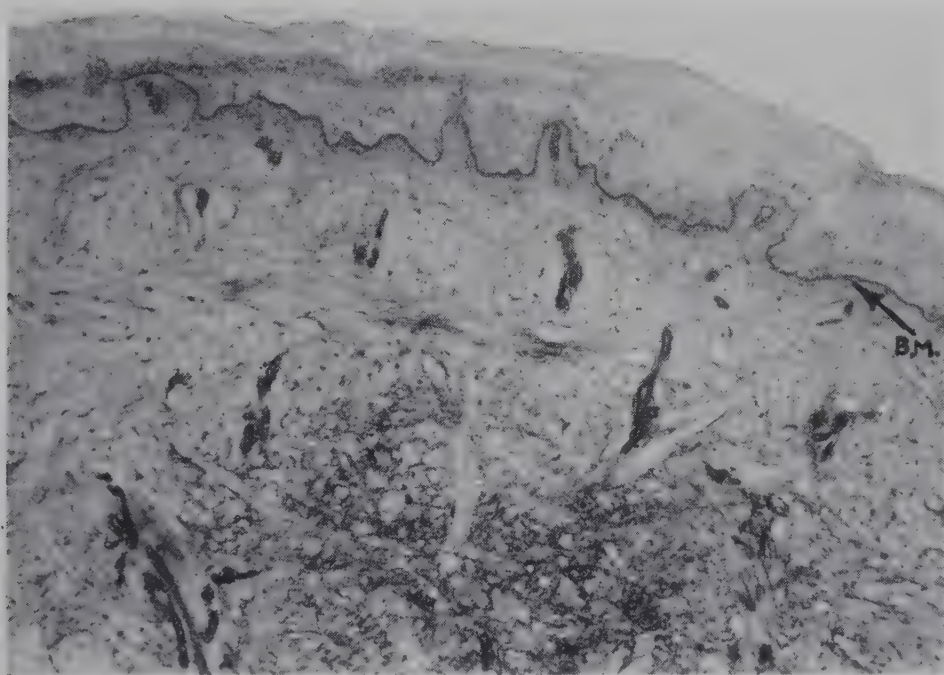


FIG. 4.—Section of skin in pretibial myxedema, showing the basement membranes at the dermal-epidermal junction (B.M.) and in the capillary walls and mucin in the mid-corium. P.A.S. stain. $\times 125$.

smooth again. There was an increase in basal metabolic rate following cortisone or ACTH therapy, but no increase in the uptake of radioactive iodine was observed.

In myxedema there is a very viscous joint fluid. Ropes (254) gives figures for knee joint (Table 6). "*Pretibial myxedema*" is a

moved by hyaluronidase in vitro and temporarily in vivo. Figure 4 shows a section from a case of recently treated Graves's disease with bilateral pretibial myxedema; the biopsy was made from a thick plaque on the shin. The basement membrane at the dermal-epidermal junction and those about the

small blood vessels have been stained with P.A.S.; the mucin is seen as dark-staining fibrillary and vacuolated material in the mid-corium between the connective-tissue fibers. This latter metachromatic material was easily removed with testicular hyaluronidase; but incubation with this enzyme had no effect upon the P.A.S.-positive basement membranes.

The local accumulations of mucin and bound water over the shins is similar to the general accumulations in the skin in generalized myxedema. Watson and Pearce (291) obtained large specimens of pretibial myxedema and carried out alkaline extraction of the interfibrillary mucinous materials and compared them with normals from amputated legs (Table 7).

were compared, and in the thyroidectomized group treated with thyrotropic hormone there was an increase in water and in glucosamine-containing ground substance, while there was no difference in the dry weight of the tissue. The abnormal periocular tissue was strongly metachromatic, and the metachromatic material could be removed with hyaluronidase.

Though the thyrotropic hormone may be the main factor in producing these changes, it is difficult to account for the peculiar distribution of lesions which involve the periocular connective tissues and the pretibial skin; and one may turn to the postulate of Gersh and Catchpole (96), that there are regions or families of cells, such as fibroblasts, peculiarly susceptible to hormone stimulus,

TABLE 7

	WATER CONTENT (PER CENT)	ASH (PER CENT)	FREE ACID (MG. PER 100 GM. OF FRESH SKIN)	
			Hyaluronic	Chondroitin Sulfuric
Normal skin.....	61.0	0.74	24.5±5.7	26.2±4.7
Pretibial myxedema.....	74.8	0.45	63.6	48.7

It seems from this work that both the hyaluronic acid and the chondroitin sulfuric acid of the skin are greatly increased in pretibial myxedema.

Commonly, patients with pretibial myxedema also have marked proptosis, which is probably due to accumulation of mucin and bound water in the connective tissues of the orbit. The experimental work of Ludwig, Boas, and Soffer (164) suggests that the mechanism depends on an excess of thyroid-stimulating hormone in the absence of normal thyroid secretion. These workers treated thyroidectomized guinea pigs with thyrotropic hormone (extract of adenohypophysis), after which these animals had marked proptosis, whereas thyroidectomy alone produced no such effect and thyrotropic hormone in the intact animal gave only slight initial proptosis. The excised orbital tissues

as, for instance, the fibroblasts at the symphysis pubis of the guinea pig, with their selective response to relaxin in pregnancy, or the connective-tissue cells in the cock's comb or in the "sex skin" of monkeys under the stimulus of sex hormone.

B. SEX HORMONES

The extracellular accumulation of water, mucopolysaccharide, and protein in the sex skin of the monkey (p. 382) has some resemblance to the lesions in pretibial myxedema. Zuckerman *et al.* (304, 217) showed that from this sex skin a viscous fluid could be squeezed and that this fluid had a water-binding effect greater than that of plasma, though the protein content was only 2.5 per cent. With acetic acid a stringy mucin was deposited from the tissue juice. Such mucin could not be detected in the same skin at

other phases of the estrus cycle, nor could it be found in the blood stream at estrus, and it was suggested that the mucin was secreted by the connective-tissue cells. Zuckerman (304) injected monkeys with estrone in the postovulatory phase of the estrus cycle and produced such swellings of the sex skin as would normally be present only in the pre-ovulatory phase. Chain and Duthie (42) found that the mucin from monkey sex skin could be rapidly reduced in viscosity by hyaluronidase; and Duran-Reynals (73) demonstrated local reduction of sex-skin turgor by the injection of hyaluronidase, showing at the same time removal of metachromatic material. Bachman *et al.* (8) transplanted skin from the genital area to other sites and found that the same skin responded to estrogenic hormone in its new site. This seems further to support the idea of a particular response of connective-tissue cells belonging to certain sites of the body. The continuance of estrone therapy for over a month resulted in response of areas beyond the genital and a gradual extension of the "pachydermatous edema," implying that sufficient stimulus would produce the same response in less specialized connective-tissue cells. These authors noted that sympathectomy had some effect in reducing the sex-skin response, but what was mainly altered was the vascular component that gives the lurid color to the genital skin at estrus.

Seeberg (260) compared intradermal reactions in women at different phases of the menstrual cycle: On the first day of menstruation or on the preceding day, diffusion in the skin is more rapid than 12 days after menstruation. At menstruation a saline bleb would diffuse away faster; P^{32} as an indicator injected into the skin would diffuse away faster; and the tuberculin type of reaction would be depressed, owing to diffusion away of the allergens.

Lurie (165) finds that the spread of tuberculosis in rabbits depends upon resistance at the portal of entry and that this is modified by the permeability of the connective tissue. This permeability is reduced by estrogens, which increase the turgor of the ground sub-

stance and reduce the spread of India ink. According to Lurie, the spread of tuberculosis and of vaccinia virus and of streptococcal and pneumococcal infections is reduced by estrogens. Luteinizing hormone or gonadotropin has an opposite effect and renders the ground substance more permeable and favors the spread of organisms. Estrogens also seem to reduce permeability of small blood vessels, while gonadotropic hormone has the opposite effect. Sprunt (270) finds that spread in the ground substance during pregnancy and under the effect of estrogenic hormones is reduced and that the reverse holds for gonadotropic hormone; and he considers that these factors influence the spread of infecting organisms. Catchpole (39) described "depolymerization" of the ground substance in the stroma of the rat ovary under the influence of gonadotropin (from pregnant mare's urine).

The comb of the cock owes its turgor in part to a zone of loose connective tissue between the vascular core and the compact layer of connective tissue on the outside. Castration, besides reducing the vascularity of the comb, prevents the formation of the mucoid layer (116); and the injection of testosterone causes the connective-tissue cells to secrete mucin again. Ludwig and Boas (163) obtained similar results and correlated the increase of metachromatic material (identified by Boas [27] as hyaluronic acid) with increased activity of the fibroblasts under androgen stimulation. Estrogen, adrenal cortical extract, and cortisone did not favor the increase of metachromatic material in the cock's comb (28, 259). The laying hen has a similar intermediate zone of mucinous connective tissue in the comb, though it is less spectacular; and in the capon the comb will respond to estrone and to testosterone (116).

C. CORTISONE AND ACTH

Opsahl (221) found that the intradermal spread of India ink in the skin of the mouse was inhibited if cortisone was given either subcutaneously or intraperitoneally. Saline with and without hyaluronidase was used

for the India ink injections, and the results were recorded after 1 hour. If the mice were kept in a high environmental temperature (220), the spreading reactions were reduced, and cortisone had no additional effect, implying that the stress reaction had already produced a maximal cortisone-like response by the body. Menkin (177), in experiments with intravenous dyes and injection into rabbit skin of leukotaxin (p. 100), found that extract of adrenal cortex inhibited the inflammatory response as measured by exudation of dye. Menkin suggested that the adrenal cortical hormone played a part in controlling capillary permeability. This may well be the main factor in the kind of experiment described by Opsahl, and we may be able to discard the speculation about the antihyaluronidase effect of cortisone, when anyhow the role of hyaluronidase in the skin may be nonexistent. Cortisone acetate or hydrocortisone acetate injected into the inflamed joints of rheumatoid arthritis after the removal of the synovial fluid (59) results in the appearance of synovial fluid of less volume and higher viscosity, together with symptomatic relief. There is no increase in hyaluronic acid as judged by the hexosamine content of the synovial fluid, and the sugges-

tion is that the steroid has an anti-inflammatory effect rather than any specific effect on the hyaluronic acid.

Seifter *et al.* (263, 262) found that cortisone would abolish the effect of hyaluronidase in increasing the permeability of rabbit bladder membrane and lens capsule; but testosterone and cholesterol had a similar effect, whereas desoxycorticosterone acetate (DOCA) enhanced the effect of hyaluronidase in the isolated-membrane perfusion experiments.

Winter and Flataker (296) demonstrated, in both normal and adrenalectomized animals, an inhibition of the spreading reaction in the skin by cortisone, adrenal cortical extract, and testosterone propionate. DOCA had no such inhibitory effect. The same authors also used the pressure required to make intradermal injections as a measure of the state of the ground substance, claiming that prior treatment of the animal with cortisone altered the ground substance of the skin so as to require a greater pressure to effect injection of a standard amount of saline (297), while other steroids had no such effect. There does not appear to be sufficient evidence for ascribing these changes to primary effects upon the ground substance.

VI. CONNECTIVE-TISSUE REPAIR

A. THE GROUND SUBSTANCE IN WOUND HEALING

In wound healing and in hyperplastic inflammatory reactions following the earlier exudative and vascular phases, fibroblasts appear from the wandering, undifferentiated connective-tissue cells, often in close proximity to the capillary walls. New vessels grow out from the pre-existing ones, and fresh ground substance can be seen around the proliferating fibroblasts. Wonderful observations on this stage of repair were made by Stearns (272, 273), with day-by-day examination of repair tissue under high power in the transparent chamber of the rabbit's ear. Invasion of the field by fibroblasts takes place about 6 days after the operation for insertion of the chamber and goes on

pari passu with vascularization. However, fiber formation was also observed independent of vascularization, especially in overgrowth of the cover slip in the moat preparation, where fibrillogenesis could be seen under conditions close to tissue culture. After fibroblast multiplication Stearns observed that many fibroblasts contained refractile granules in the cytoplasm; and another observation was the bubbling or vesication from the fibroblast surface, after which fibers would form quite rapidly over a few hours. She thought that actual fiber formation was extracellular, though directly related to secretion by fibroblasts.

Gersh (96) also noticed granules in the fibroblasts in relation to wound healing and found that these granules were P.A.S.-posi-

tive and therefore suggested that they contained mucopolysaccharide and that this material was contributing to the formation of ground substance. During wound healing the ground substance of the skin increases and is found to be metachromatic and to contain an excess of P.A.S.-positive material. Bensley (20), in the study of guinea-pig pancreas fibrosis, noted, first, edema of the stroma, and then, at about 10 days from ligation of the pancreatic duct, fibroblast increase and the ground substance becoming viscous and metachromatic. Argyrophil fibers then form, which later merge with collagen. Bensley found that mucinous material could be extracted from the ground substance in healing granulation tissue. Sylvén (277) drew attention to metachromatic material in healing wounds and claimed that this material was mucopolysaccharide. Mast cells and some other connective-tissue cells also contain metachromatic material; but it may be noted that hyaluronidase will remove metachromasia from the ground substance but not from the mast cells or fibroblasts.

Layton (152) has shown that embryonic tissues, granulation tissue, and healing wounds have a high capacity for fixing inorganic sulfur both *in vitro* and *in vivo*. Sulfate labeled with S^{35} in special incubating solutions is fixed by healing tissues and by embryonic tissues but not by adult muscle or skeletal tissue in the undamaged state (153). The sulfate is probably incorporated into chondroitin sulfate in the formation of ground substance, and this may be an important step in healing.

B. SCURVY

The failure of sound wound healing in scurvy seems to be related to inadequate production of connective tissue, in which an abnormality of the ground substance may be a factor.

Wolbach (299) and later Penney and Balfour (227) studied the repair process in blood clots in guinea pigs. In the normal animal vascular invasion of the clot is seen within about 4 days of injury, and by the

fifth day a syncytium of fibroblasts has appeared. Extracellular metachromasia is seen around the fibroblasts, indicating the production of amorphous ground substance, in which reticulin soon appears and, finally, collagen fibers in the same site. In the scorbutic animal new capillaries invade the clot but fail to canalize; and, while fibroblasts multiply and invade the clot, too, their appearance remains embryonic. There is failure to form metachromatic ground substance, reticulin is scanty, and collagen fiber formation does not take place. Within 6–12 hours of giving vitamin C, metachromatic material appears; and cell response, vascularization, and fibrillogenesis all become normal.

Fell and Danielli (82, 53) found a low alkaline phosphatase activity for repair connective tissue in the skin wounds of scorbutic guinea pigs, and they demonstrated a correlation between graded low vitamin C intake, alkaline phosphatase in the connective tissue of wounds, and fiber organization. French and Benditt (90) have recently found that alkaline phosphatase in healing wounds is increased if epithelium is present in the repair area. There has been some uncertainty as to whether vitamin C affects fiber formation in tissue culture. Hass (119) attempted to exclude vitamin C from the culture medium and claimed that this had no effect upon fiber formation by growing fibroblasts. On the other hand, von Jeney and Törö (137) found that vitamin C would promote fiber formation in tissue culture.

The absence of metachromasia in scorbutic wound healing might suggest a failure of the usual production of ground substance; however, Gersh and Catchpole (96) found the extractable mucopolysaccharide in the ground substance of wounds in scurvy to be greater than in wound tissue of normal animals. It was suggested here that the ground substance was in a state of "depolymerization," in which state it would be more easily soluble in water and would not give metachromasia. Pirani (232) thought that the high serum glycoprotein in scorbutic guinea pigs might be related to the breakdown of

connective-tissue ground substance, with absorption into the blood stream of derivatives of the ground substance. This is somewhat speculative, as there is no clear chemical relationship between the known ground-substance mucopolysaccharides and the blood glycoproteins. There is no breakdown of already formed collagen in scurvy, and Elster (79) estimated the collagen content of the organs of scorbutic animals to be the same as that of normals. Orekhovich (223) found values of citric acid-soluble "procollagen" (p. 409) to be lower in scurvy than in either normal animals or starved animals, and it was thought that this indicated diminished connective-tissue synthesis.

Reppert *et al.* (244) claim that vitamin C has an inhibitory effect upon spreading induced by hyaluronidase in the skin, and they suggest that vitamin C may have a direct effect upon the cement substance of the capillaries or upon a balance between hyaluronic acid and hyaluronidase. But the evidence is insufficient. The exact mechanism of the defect in scurvy is not clear, but, according to Klemperer (145), the experiments under discussion "tend to show that not only normal fibroblasts but also a homogeneous ground substance, adequate in amount and in chemical or physicochemical constitution, is of paramount importance for the formation of collagen fibres."

As scurvy develops in the guinea pig, there is some adrenal hypertrophy and increase of 17-ketosteroid excretion which can be reversed by cortisone; but, beyond this, ACTH and cortisone have no effect on the manifestations of scurvy in these animals (49, 14), and this is also true of scurvy in monkeys (274). In fact, there is no definite correlation between vitamin C action and cortisone in wound repair, in spite of the storage of vitamin C in the adrenal and in contradiction to earlier experiments with cortisone in scurvy (241).

C. THE EFFECT OF ACTH AND CORTISONE ON HEALING

Many observations have been made upon the effects of cortisone and ACTH upon

wound healing. Ragan (242) showed that experimental skin wounds in cortisone-treated rabbits showed delayed healing; and Spain (268) found that, in wounds where cortisone delayed healing, there was low cellularity, no new capillary formation, sparse fibroblast proliferation, and absence of metachromasia of the ground substance, but that epithelialization would proceed normally. Additional ascorbic acid had no effect on the wound healing here, nor was inhibition an effect of protein imbalance (235). Animals differ in the extent to which cortisone affects them; and cortisone, for instance, has no effect upon wound healing in the guinea pig (287), while that in the rabbit is markedly affected. Taubenhause and Amromin (281) studied granulation tissue in the walls of turpentine abscesses in growing rats. In animals treated with DOCA the walls of the abscesses had numerous large capillaries and many large fibroblasts with P.A.S.-positive cytoplasmic granules, while animals treated with sex hormones had reduced granulations and poor healing. Pirani (234) sampled healing wounds in guinea pigs receiving DOCA and found an excess of granulation tissue in the wounds, while mature connective tissue was unaffected. Shapiro (265) injected turpentine into two sites of the same animal, adding cortisone to one injection and cholesterol to the other. Only in the inflammatory area where cortisone had been introduced was there suppression of the granulation-tissue response, showing in this instance that the effect produced by cortisone was a local one. He further showed that denervation and moderate ischemia had no effect upon this reaction.

Topical application of an alcoholic solution of adrenal extract, besides delaying wound healing, was found to reduce granulation tissue, with inhibition of proliferation of fibroblasts and vascular endothelium. Delay in the appearance of fibroblasts and prolonged immaturity of the collagen were noted in nonpenetrating wounds of the cornea (14) whether the cortisone was locally applied or given systemically. Dougherty (66) showed a correlation between local utiliza-

tion of cortisone and the degree of experimental inflammation, and he stressed the local "antiphlogistic" action of cortisone. Spain (267) showed that healing of skin wounds in mice was depressed if cortisone was given in doses comparable with what is given therapeutically in man. In human skin wounds, Creditor *et al.* (51) showed histologically that cortisone delayed healing, with inhibition of granulation tissue, in spite of overgrowth of epithelium. Injection of hyaluronidase had no effect on this inhibition of healing, and it seemed unlikely that cortisone was acting in any sense as an antihyaluronidase.

Castor and Baker (38) found that prolonged local application of alcoholic solutions of cortisone and compound F to the skin of the rat resulted in cessation of hair growth, diminution in size of sebaceous glands, thinning of the dermis, and lessening of the number of connective cells. This again emphasizes species differences in response to steroids, as no such effects are seen in man.

The early phase of inflammatory reaction in the skin as produced by locally applied irritants (192) or by burns (195) is markedly reduced by cortisone. During the first 24 hours the red cell diapedesis, capillary damage, and edema are delayed by cortisone. An effect upon the capillary walls is considered to be of primary importance.

Experiments purporting to show that cortisone has a direct antihyaluronidase effect in connective tissue owe much of their misinterpretation to failure to allow for the effect of cortisone upon the small blood vessels, both in repair processes and in the more acute phases of inflammatory reactions. It is clearly seen that cortisone prevents the vascularization of the cornea (138). Ashton and Cook (7) followed the effect of cortisone on vascularization of wounds in the rabbit's ear as seen through the transparent chamber, and they attributed delay in wound healing to the vasotonic effect of cortisone on the capillaries. Ebert and Wissler (75) studied more acute reactions through the transparent chamber in the rabbit's ear and found that cortisone reduced the vasodilatation

and endothelial damage of hypersensitivity reactions to horse serum. Recent work by Ebert (74) suggests that the cortisone effect may be modified by the initial caliber of the blood vessels; for, experimentally, cortisone, besides raising the threshold response of the vessels in the rabbit's ear to the vasodilator effect of histamine, will also raise the threshold response of these vessels to the constrictor effect of nor-adrenaline. Levine (160) finds that, in the adrenalectomized animal, adrenal cortical extract will restore the responsiveness of small blood vessels to nor-adrenaline (p. 103); this mechanism was thought to be of great importance in the fatigability and exhaustion of muscle in the adrenalectomized animal. The response of the intact animal to cortisone may be different, and different regions of the body may respond in different ways, but there seems to be little doubt that, by some indirect action, cortisone has an important effect upon the tonus of small blood vessels. It may then appear indirectly to affect the spreading reaction in the skin.

Taubenhaus and his associates (282, 283) have examined the effects of hormones on granulation tissue in some detail, using the preparation already mentioned, namely, the wall of the turpentine-produced abscess in the rat, both intact and after hypophysectomy. Hypophysectomy results in poor production of granulation tissue and poor healing, and this is reversed by growth hormone from the anterior pituitary. Growth hormone also increases granulation tissue in the intact animal and reverses the depressant effect of sex hormones on healing; and it is suggested that sex hormones may owe their effect on granulation tissue to inhibition of release of growth hormone. The effect of growth hormone is not only to increase the amount of granulation tissue but also to promote healing; that is, besides increase in size and number of fibroblasts, there is increase in the formation of collagen fibers. It was shown that, while DOCA would increase granulation tissue, this tissue remains primitive in appearance, with immature fibroblasts in an abundant ground substance,

while fiber formation is retarded. DOCA has to be given in advance before the experimental wound has been made, and it is considered that its effect may be an indirect one, perhaps resulting from electrolyte changes.

It would be interesting to know whether cortisone has any effect upon the growth of fibroblasts. Blunt *et al.* (26) found that the fibroblasts in healing wounds under the influence of cortisone showed morphological changes. Barber and Delaunay (15) found cortisone to have an inhibitory effect on the growth of fibroblasts from chick embryos in tissue culture; but it should be noted here that enormous amounts of steroids were used. Baldridge *et al.* (11) found cortisone to have no effect upon the growth of chick fibroblasts in tissue culture. Porter (237) suggested that there was no change in migration or proliferation of fibroblasts under the direct influence of cortisone but that there was a failure of fibrillogenesis; and he also thought that compound F inhibited fibrillogenesis. Chick embryo tissues and healing tissues normally take up inorganic

sulfur strongly; but, according to Layton (154), cortisone inhibits this. Layton finds that with amounts of cortisone that inhibit sulfur uptake there is still no effect on the migration of fibroblasts. Young rat littermates were studied (155) under conditions of controlled sulfur balance, and animals receiving cortisone were found to be in negative sulfur balance. Layton gives a comparison of fixed sulfate in the skin (Table 8).

TABLE 8

	Chondroitin Sulfate, Mg. per Gm. Dried Tissue
Cortisone-treated.....	1.6 \pm 0.5
Controls.....	6.2 \pm 0.3

There is close correlation here between the uptake of sulfate, which is evidently concerned with the formation of the chondroitin sulfate of ground substance and the formation of collagen fibrils, just as the formation of metachromatic ground substance and that of reticulin and collagen in healing wounds were noted to be closely related.

VII. THE DEVELOPMENT OF GROUND SUBSTANCE

A. ORIGIN OF GROUND SUBSTANCE

A viscous intercellular substance is present very early in embryonic development. Baitsell (9) studied serial sections of chick embryo tissues and examined live chick embryos under the dissecting microscope. In the early embryo the forerunner of the connective tissue is a transparent, jelly-like, cell-free ground substance which fills the space between ectoderm and endoderm; it is only later that this space is invaded by mesenchymal cells which have migrated from the primitive streak. During further development of the embryo the ground substance is believed to be secreted by the stellate cells of the mesenchyme (175).

While nearly all the cells of the body may contribute to the medium between cells, we have to consider the special accumulations of ground substance in connective tissues and their relation to the fibers. In 1861 Kölliker summarized the currently held view

that the ground substance was probably secreted by the connective-tissue cells, but he allowed the possibility of an independent source of ground substance (148). He considered that even though the ground substance might not be formed in the connective-tissue cells, these cells influenced both the formation of ground substance and the laying-down of collagen fibers. Today the question of these exact relationships is still unanswered, but most investigators would agree that the activity of fibroblasts, the laying-down of ground substance, and the formation of collagen fibers are closely interrelated. Meyer (182) suggests that mucopolysaccharides and native soluble collagen are both secreted by fibroblasts and that the acid mucopolysaccharides play a part in shaping the fibrous protein.

The raw materials from which the various connective-tissue elements are formed are presumably derived from the plasma, the

proteins of which may contribute to the amino acids which go to build up the connective-tissue proteins (84). If proteolytic enzymes are concerned in this process, which involves transpeptidization (92), it appears that acid mucopolysaccharides in binding water may be instrumental in allowing protein synthesis to proceed (248).

Workers with tissue cultures of fibroblasts have argued about whether reticulin and collagen fibers are formed within the fibroblast or whether they are organized in the medium after precursor substances have been secreted by the cells; but most workers agree that fibroblasts are essential to collagen formation, and the field has been reviewed by Porter (237). He finds that it is possible to produce collagen from fibroblasts in media lacking blood and other tissue components (modified Locke's fluid), suggesting that fibroblasts contain all the necessary material for forming connective tissue, though the fibrils mostly have axial periods of 220 Å instead of the 640 Å of better-differentiated collagen. He noticed that there was some nonfibrous material which he thought might be mucopolysaccharides of ground substance and that this merged with collagen and might be contributing to its formation.

Fetal connective tissue contains much mucopolysaccharide, which shows variable metachromasia, and, although the subcutaneous tissue is strongly metachromatic, the dermis usually is not (298). The mucopolysaccharides may be extracted with water, and it is important to distinguish glycogen, which is also plentiful in fetal ground substance. Gersh and Catchpole (96) note a gradual change from the primitive ground substance of the rat at birth to a less soluble, more highly polymerized state as the animal ages, with increase of fibers and gradual differentiation of basement membranes at the junction of connective tissue and epithelial and endothelial structures.

In healing wounds a stage is seen at which the fibroblasts have increased in the repair area and an accumulation of metachromatic ground substance is present (96, 32). At a

later stage, when collagen fibers are being laid down, the ground substance diminishes. This relationship between fibroblast activity and ground-substance accumulation is also seen in the mucinous accumulation of monkey sex skin (73), in the developing cock's comb (163), and in productive inflammation (1). Some of the fibroblasts in areas of repair contain granules, the staining properties of which suggest that they may contain mucopolysaccharides (96), which are being secreted into the ground substance (169).

B. FIBRILLOGENESIS AND RECONSTITUTION OF FIBRILS

Certain kinds of collagen (rat-tail tendon, fish swim bladder) can be dissolved in acetic acid, to yield clear viscous solutions (p. 394) from which fibrous proteins can be reprecipitated with saline (214, 292). Reconstituted fibers studied with the electron microscope (288) show either the axial periods 640 Å characteristic of collagen or no characteristic striations, depending upon salt concentration and other environmental factors. Gross (109) finds that fibers reconstituted from ichthyocol (from connective tissue of the carp swim bladder) cannot be obtained free of carbohydrate. The acetic acid extract was thrice purified by cathodal electrode deposition, and yet fibers reprecipitated by salt were not free of hexosamine.

Orehovich *et al.* (224) found that compact collagen, such as that from the dermis, could be dissolved in a citrate buffer of pH 3–4.5, giving a viscous, clear extract of "procollagen," which would crystallize on dialysis against water (p. 409). Highberger and Gross (128) examined the conditions under which fibers could be reprecipitated from procollagen and studied these fibers under the electron microscope. They found fibers with the characteristic 640 Å cross-striations of collagen and, in addition, fibers with repeating axial periods of 2,000–3,000 Å, which they called "long-spaced fibrils." These long-spaced fibrils are not found in nature and are thought to be due to an excess of mucopolysaccharide affecting the reprecipi-

tation of collagen fibrils, as this type of fibril predominates if mucopolysaccharides are added to procollagen. It has been suggested that a mucopolysaccharide, such as heparin (197), may be essential to the formation of collagen fibers because of its effect on procollagen precipitation; but the evidence is insufficient to support this view. Wyckoff (303) in describing the fine structure of collagen under the electron microscope differentiates between the disklike 640 Å elevations and the tiny cross-threads sometimes seen running over these larger periodic elevations. Two or occasionally three such cross-threads could be seen in clean specimens, and he thought that these fine details became visible only when adherent material, probably mucopolysaccharide, had been removed from the fibers.

Little is known of the structure of elastic fibers, and they lack the periodic cross-striations that distinguish collagen under the electron microscope (302). There is evidence of a close association between the fibrous protein of elastin and a special cement substance which has been called "elastomucin" (114). Elastic fibers dissolved by pancreatic elastase (12) yielded a pure preparation for chemical study (114), and the elastic fiber was thought to have a core of fibrous protein "proelastin" surrounded by an outer coat of elastic protein combined with polysaccharide and sulfate belonging to the elastomucin, or cement.

It has been suggested (180) that, in the skin, fibroblasts secrete the mucopolysaccharides of the ground substance and the precursor materials of connective-tissue fibers, though additions are probably made from the interstitial fluid.

It is possible that in certain circumstances other cells may secrete hyaluronic acid. Tissue cultures from the synovium of the rabbit knee joint (290) grew cells morphologically distinct from fibroblasts, and these synovioblasts had varyingly metachromatic cytoplasmic granules. These cells form mucin and also contain a fibrinolytic ferment. The culture may revert to fibroblast form, in which case it stops producing

mucin. Meyer (179) describes how hyaluronic acid was isolated from a synovioma not only at its original site but also from a metastatic deposit in the liver.

The skin is capable of rebuilding its mucopolysaccharide fairly rapidly, and McClean (200) noticed that the effect of testicular extract on the spreading of dye in the skin would wear off after between 24 and 48 hours. Bywaters (37) found some variation in the time of recovery of the skin with age, but the usual time to restore the barrier to spreading after hyaluronidase had been injected was 24–48 hours. Hemoglobin was used as an indicator of spreading in the skin. The time for reconstitution of the dermal barrier was prolonged in rheumatoid arthritis and in acute rheumatic fever, and the authors suggested that this might be in some way related to the pathogenesis of these diseases.

C. MAST CELLS

Asboe-Hansen (4) brings forward some evidence for the view that hyaluronic acid is secreted by the mast cells of Ehrlich from a heparin-like precursor. His main argument is that wherever there is a notable accumulation of mucopolysaccharide, there will be found numerous mast cells, as in the umbilical cord (238, 136), in synovial membrane (5), in the eye, and in myxedema cutis. He states that the pituitary thyrotropic hormone increases the number of mast cells, whereas thyroid extract reduces mast cells and connective-tissue mucin, and cortisone reduces the number of mast cells (6, 40). Asboe-Hansen (5) finds that injection of hyaluronidase into the skin removes hyaluronic acid and leads to the discharge of granules from mast cells but that, after 24–72 hours, re-formation of large mast-cell granules is seen prior to reconstruction of the dermal hyaluronate. All will not agree that the number of mast cells in granulation tissue, for instance, is impressively increased, and at present there is insufficient evidence for ascribing the role of hyaluronate formation to mast cells. As long as the granules in mast cells and in fibroblasts are both variously metachromatic and P.A.S.-

positive, some of us may have difficulty in deciding which cell is which.

Jorpes (140, 139) established the mast cell as a source of heparin. He finds that the heparin is in the form of a monosulfuric acid ester and that, after release from the cell, it may be in the form of di- and trisulfuric esters. It is in the more highly sulfated form that the heparin is active as an anticoagulant and more freely soluble in water. The more sulfated forms, however, are no longer P.A.S.-positive, while the monosulfuric acid ester as found in the mast cell has available groups for oxidation with periodic acid. This mast-cell heparin has equal parts of glucosamine, uronic acid, and sulfuric acid. Acid hydrolysis of this material is slow compared with hyaluronic acid and chondroitin sulfuric acid. Sylvén (279) used differential extractions of mast-cell cytoplasm to show that the heparin was not in the coarse granules but was present as an amorphous coating. He thought that the heparin here was probably linked to lipoprotein.

Mast-cell tumors are fairly common in the skin of dogs and show varying grades of malignancy. Bloom and his associates (225) succeeded in cultivating mast cells from such a tumor *in vitro*. Mast cells grew out in sheets and appeared to inhibit the growth of other cells. Metachromatic granules were seen, and amoebic movements of the mast cells could be followed. Oliver (219) had been able to extract large amounts of physiologically active heparin from mast-cell tumors of dogs, in which the amount of heparin obtained would depend on the degree of differentiation of the tumor. Evidently, the heparin remains inactive as long

as it is within the cell, as these animals have no disorder of blood coagulation. Bloom (25) found that a malignant mast-cell tumor of the dog regressed both macroscopically and microscopically under the influence of cortisone. Ehrich and his associates (77) report the interesting case of a man with enormous elephantiasis of the scrotum. The excised tissue was found to contain very many mast cells around the small blood vessels, and from this tissue they were able to extract heparin, which would act as an anticoagulant in animals. They obtained 126 mg. purified heparin per kilogram of scrotal tissue. It is to be noted here also that the man had no blood-clotting disorder.

Drennan (67) studied mast cells such as occur under the mucosa of the tongue and esophagus in the human, and, while they all contain metachromatic granules, comparatively few of them are P.A.S.-positive. However, the mast cells of the skin in urticaria pigmentosa are nearly all P.A.S.-positive* (probably because the heparin is present as monosulfate ester). If the skin of an urticaria pigmentosa lesion has been stimulated just before biopsy is performed, the mast cells will have discharged their granules, becoming swollen and diffusely metachromatic or burst and shrunken. It is suggested that in the presence of edema the mast cell imbibes fluid (the heparin being strongly hydrophilic), and the cells swell and may burst. The recent demonstration of histamine in tissues with high mast-cell content¹ is of very great interest, and it seems probable that mast cells release histamine as well as heparin.

VIII. GROUND SUBSTANCE IN SOME PATHOLOGICAL CONDITIONS

A. CONNECTIVE-TISSUE CHANGES IN AGING SKIN

The most obvious change which takes place in the skin with aging is loss of elasticity; though this may be partly due to changes in the fibrous connective tissue, it is mainly the hydration of the dermis that is altered. The extensibility of the skin is

largely related to its state of hydration (253) (p. 6), and this, in turn, is probably related to its content of mucopolysaccharide, of which hyaluronic acid is powerfully hygroscopic. Halliburton (115) pointed to a higher

1. J. F. Riley and G. B. West, The presence of histamine in tissue mast cells, *J. Physiol.*, **120**:528, 1953.

mucin content in the skin of children as compared with that of adults, but no satisfactory chemical data are available. In animals there is progressive diminution of skin hydration with aging. Gersh and Catchpole (96) found much water-soluble ground substance in the skin of the newborn rat; but with aging there was a progressive loss of water-soluble ground substance, and fibers and basement membranes appeared to differentiate at the expense of the ground substance. Orekhovich (223) found a diminution of "procollagen" extractable from the dermis with increasing age in guinea pigs, and he related this to diminution in fibroblast activity and to diminished fibrillogenesis in general.

A particular change in the exposed skin of old people is the so-called "senile elastosis," in which the connective tissue in the subpapillary corium shows relative basophilia and staining with acid orcein. Unna (286) thought that this was a merging of collagen with elastic fibers. In senile skin Percival (228) found the collagen fibers to be elongated and tortuous, with these degenerate fibers containing fat globules. Recent studies with the electron microscope (285) revealed in this degenerate connective tissue much amorphous material which may belong to the ground substance merging with fibers lacking the cross-striations of collagen and showing abnormal kinking, together with some normally striated collagen fibers of the same diameter. The degenerate collagen fibers were susceptible to digestion with trypsin. There is evidently no increase in elastic fibers in this condition, in spite of the staining reaction, the change being a marked accumulation of amorphous material of ground substance merging with degenerate collagen fibrils. Gross (108) examined the electron microscopic appearance of the skin of the rat from the point of view of aging. In the newborn animal there was a large amount of amorphous material (ground substance) which was inclined to obscure the fibers of collagen, but it could be easily washed away with water. With older

skins there was less amorphous ground substance clinging to the fibers, but such as was present was more difficult to remove. In these studies the collagen fibrils showed the same essential striation at different ages, though in older animals the collagen fiber bundles had a greater average diameter than in the younger ones. Gross found the same difference between infant and aged human skin (107).

It is not always the hydration of the ground substance that is the main factor in elasticity. The extreme elasticity of the skin in Ehlers-Danlos syndrome is one instance in which the elastic fibers themselves are greatly increased in number, and Tunbridge (285) has shown that under the electron microscope these are indeed elastic fibers. It should be mentioned that collagen fibers, while having little intrinsic elasticity, do allow of reversible stretching through their organization into flexible networks (p. 6).

B. MUCOID DEGENERATION AND FIBRINOID DEGENERATION

Pathological changes in the ground substance are insufficiently well understood to allow of interpretation in terms of altered functioning of the connective tissue. Great interest has been focused upon the group of diseases that Klemperer, Pollack, and Baehr (146) characterized as having systemic alterations in the connective tissue, namely, systematized lupus erythematoses, rheumatic fever, rheumatoid arthritis, scleroderma, dermatomyositis, serum sickness, and polyarteritis nodosa. Interesting reviews of this field of pathology are those of Klemperer (145) and Ehrlich (76). Commonly seen in these diseases are, first, mucoid degeneration, which may depend upon an increase or change in the mucopolysaccharides of the ground substance, and, second, fibrinoid degeneration or necrosis. This latter pathological change is thought to represent a combination of protein (derived either from local tissue damage or from the blood) (103) with mucopolysaccharides of the ground substance (1). Either type of

degeneration may be followed by hyalinosis or sclerosis. None of these connective-tissue changes is specific for the diseases mentioned, for they may be found in all manner of damage to connective tissue, but their prevalence in the so-called "connective-tissue diseases" is emphasized. It is hardly justifiable to suggest a disorder of collagen, on the one hand, or of ground substance, on the other, as so little is known of the normal physiology of fibers and of ground substance, although what is most evident is that they are closely interdependent. For instance, in scleroderma, where alteration of collagen fibers has been considered to be the most striking pathological change, examination of the diseased skin under the electron microscope reveals an excess of amorphous material probably belonging to the ground substance, with no obvious qualitative abnormality of the collagen fibrils (264); and the same may be true of other conditions formerly grouped together as "collagen diseases."

C. AMYLOIDOSIS

The most striking changes in the ground substance are seen in amyloid disease, which is remarkable for extracellular accumulation of metachromatic material. Hass (117) has, by differential extraction, shown amyloid to consist mainly of protein, together with $\frac{1}{2}$ –1 per cent of carbohydrate (containing hexosamine, uronic acid, acetyl and sulfate groups). Meyer (179) found the carbohydrate of amyloid to be a monosulfuric acid ester with acetylhexosamine and uronic acid; this rather resembles heparin and is not affected by hyaluronidase. Meyer thought that amyloid might be an excessive accumulation of a cement substance normally present in the connective tissue in amounts too small to be detected. Ehrlich (76) suggests that the main abnormal constituent of amyloid may be a protein (globulin), produced by some deviation in the immunity mechanism in secondary amyloidosis. Experimental work on amyloid (118) supports the clinical conception of some alteration in the immunity reaction, involving ab-

normal protein accumulation in the ground substance. In primary amyloidosis or par-amyloidosis, plasma-cell increase or actual myelomatosis is usually present, and excessive or abnormal protein synthesis by these cells is thought to be the origin of the deposits in the ground substance (76, 127, 293).

A relationship between scurvy and amyloidosis has been suggested by Pirani (232). With experimental sodium caseinate injections in mice, Teilum (284) found that ascorbic acid reduced the incidence of amyloidosis, while cortisone increased it; but he considers that these effects are not directly upon the extracellular components of connective tissue but rather upon the cells of the reticuloendothelial system and plasma cells. The suggestion that degraded ground substance from the connective tissue in scurvy may lead to high blood levels of glycoprotein (232) has already been criticized on the ground that no relationship has, in fact, been demonstrated between this glycoprotein and the connective tissue; and the further suggestion (233) that this mucoprotein may then be deposited in the connective-tissue ground substance as amyloid is open to question. Engel (81) provides an interesting parallel in considering the action of parathyroid hormone, which leads to excessive mobilization of mucopolysaccharides from bone and cartilage. A rise in serum mucoprotein is said to follow, and subsequent renal damage is thought to be the result of blocking of the renal tubules with mucoprotein, with or without the addition of calcium phosphate. But, of course, the same criticism must be applied here, the serum mucoprotein being entirely different chemically from the skeletal ground-substance mucopolysaccharides. Degraded hyaluronic acid may, however, be mobilized as a result of the injection of hyaluronidase into the skin; and Butt (34) found alteration of urinary colloids as a result of hyaluronic acid excretion and suggested that this mechanism might be of importance in connection with calculus formation in the urinary tract.

D. IMMUNITY MECHANISM AND THE CONNECTIVE TISSUE

There is no known relationship between the ground substance and the normal immune mechanism, though an early phase in allergic granulomatous reactions (76) involves accumulation of connective-tissue mucin. However, fibroblasts, along with other cells of the mesenchyme, may contribute to antibody formation (83); and Coons (50) finds that there is some evidence that an antigen may be adsorbed onto the collagen fibers, in which case the ground substance which probably coats these fibers may be involved. In this interesting work, an antigen is located by incubating tissue in a solution containing antibody conjugated with fluorescein isocyanate, so that the fluorescent antibody becomes fixed at the sites of the antigen.

Since the discovery of the pneumococcal-specific soluble substances by Dochez and Avery in 1917 (60), important immunological work has centered upon the bacterial polysaccharides such as are responsible for pneumococcal type specificity (126, 125), and it is noted that many polysaccharides function as haptens (150), conferring specificity when linked with the protein component of an antigen. For instance, the protein moiety of the somatic antigen of *Shigella shigae* combined with a polysaccharide, such as agar, would produce antibodies capable of reacting specifically with that polysaccharide (196). It has been found that hyaluronic acid when linked to protein does not behave like an antigen (144, 133, 126) or a hapten; and the same is true of natural mammalian polysaccharides, such as chondroitin sulfate and glycogen. However, Glynn and Holborow (101) succeeded in sensitizing rabbits to a combination of chondroitin sulfuric acid and β -hemolytic streptococci, and in this experiment the animals developed arthritis. Alone, neither the chondroitin sulfate nor the streptococci produced these changes. Some such mechanism of autoantibody formation might be of importance in the rheumatic diseases.

While the production of hyaluronidase by

various cocci and clostridia cannot be related to the virulence of these organisms or to the infective process (190), the production of hyaluronic acid by just those strains of β -hemolytic streptococci that are associated with rheumatic fever has attracted much attention (240). A biological confusion of the immunity mechanism may underlie the pathogenesis of some of the so-called "collagen diseases," such as systematized lupus erythematoses.

E. THE CAPILLARY WALLS

A structural and functional component of the capillary wall is the cement substance surrounding the endothelial cells and merging with the connective tissue; and if this cement is included under the heading of "ground substance," this discussion could be extended into many realms of physiology. The P.A.S.-positive staining sleeve supporting the capillary endothelium may well be a special condensation of ground substance (96, 276) similar to the basement membranes at the junctions between epithelium and connective tissue, but we have no precise information concerning its chemical structure. These vessel cements may contain complexes of chondroitin sulfuric acid—but this is purely speculative. Meyer (179) remarks that hyaluronic acid has never been demonstrated in the vessel walls and that purified hyaluronidase has no effect on them. Zweifach and Chambers (305) found the capillary walls to contain an intercellular cement substance between the endothelial cells and discovered that this cement would allow exudation if calcium ions were removed from the perfusing fluid (p. 64). Hyaluronidase introduced into the circulation has no effect upon capillary permeability, but there is an outer sheath to the capillary wall which is softened if hyaluronidase is applied by micropipette to reach the capillary wall from the outside. Zweifach and Chambers thought it unlikely, however, that this latter mechanism would be operative under physiological conditions. Other workers (80, 19) claimed that large amounts of hyaluronidase introduced into

the circulation of animals affected the capillary walls in such a way as to allow extravasation of fluid and colloidal dye into the tissues. And the fact that the giving of cortisone reversed this effect on blood vessel walls was interpreted as an "antihyaluronidase" effect of cortisone. It has subsequently been demonstrated that purified hyaluronidase has no such effect, so that the factor which caused vascular damage in the earlier experiments was evidently an impurity, possibly causing release of histamine.² The

effect of cortisone and ACTH upon the tonus of small vessels is of the greatest importance; this was briefly discussed on page 445. Robson and Duthie (250, 251) used a negative-pressure cup for measuring the capillary resistance in human skin and were able to show a raised capillary resistance under the influence of cortisone and ACTH and under the influence of stress. With DOCA there was a fall in capillary resistance, while testosterone and estradiol had no effect.

IX. BRIEF REVIEW OF FUNCTIONS OF GROUND SUBSTANCE

In the ground substance of the skin there appear to be approximately equal amounts of hyaluronic acid and chondroitin sulfuric acid (226). The hyaluronic acid is probably present in an easily dissociable complex with protein; but little is known of the proteins of the ground substance except that they are nonfibrous and contain tyrosine and tryptophane and are easily digested by trypsin, thus being markedly different from collagen. The protein is a major structural component of the ground substance of loose connective tissue, as Day has shown (56), and it is probably equally important in the ground substance of the dermis. As a hydrated gel the hyaluronic acid plays an important part in the water binding of the dermis, and this hydrated ground substance is undoubtedly important in giving the skin additional toughness, elasticity, and resilience. It may well also have a kind of lubricating function in the skin in relation to the fiber bundles, just as hyaluronic acid is what gives the joint fluid its essential lubricating property (254).³ The gelatinous ground substance acts as a barrier to the spread of infection and probably offers resistance to the spread of some tumors, though the evidence on this point is conflicting. Chondroitin sulfuric acid as a com-

plex with protein would appear to have a special role in skeletal support. Its relation to weight-bearing is described by Matthews (171), who compared the ratio of chondroitin sulfate to collagen in different parts of the same joint cartilage and found this ratio to be higher in the weight-bearing areas of cartilage than in the nonweight-bearing areas. In the skin, however, we do not know the distribution of chondroitin sulfate. It is easy to imagine that it is particularly abundant in the basement membranes, which resist hydrolysis by hyaluronidase; however, we do not have any exact information here. Meyer (189) suggests that the chondroitin sulfuric acid might act as a cation exchange resin in regulating the passage of electrolytes or binding inorganic ions.

If one considers that fluid and metabolites of all kinds must pass through the connective-tissue "barrier" between the blood vessel and the parenchyma and that it is essentially the ground-substance continuum through which everything must pass, then this would seem to be one of the most versatile media in the body, and it is remarkable that it functions so well, even though we know so little about it.

2. W. Feldberg and A. A. Miles, Regional variations of increased permeability of skin capillaries induced by histamine liberator and their relation to histamine content of skin, *J. Physiol.*, **120**:205, 1953.

3. A. G. Ogston and J. E. Stanier, The physiological function of hyaluronic acid in synovial fluid; viscous, elastic and lubricant properties, *J. Physiol.*, **119**:244, 1953.

BIBLIOGRAPHY

1. ALTSHULER, C. H., and ANGEVINE, D. M. Histochemical studies on the pathogenesis of fibrinoid, *Am. J. Path.*, **25**:1061, 1949.
2. ASBOE-HANSEN, G. Hyaluronidase action on the permeability of human skin, *Acta dermat.-venereol.*, **30**:27, 1950.
3. ———. The variability in the hyaluronic acid content of the dermal connective tissue under the influence of thyroid hormone, *ibid.*, p. 221.
4. ———. A survey of the normal and pathological occurrence of mucinous substances and mast cells in the dermal connective tissue in man, *ibid.*, p. 338.
5. ———. The origin of synovial mucin. Ehrlich's mast cell—a secretory element of the connective tissue, *Ann. Rheumat. Dis.*, **9**:149, 1950.
6. ———. The mast cell. Cortisone action on connective tissue, *Proc. Soc. Exper. Biol. & Med.*, **80**:677, 1952.
7. ASHTON, N., and COOK, C. In vivo observations of the effects of cortisone upon the blood vessels in rabbit ear chambers, *Brit. J. Exper. Path.*, **33**:445, 1952.
8. BACHMAN, C.; COLLIP, S. B.; and SELYE, H. Further studies of sex skin reactions in *Macaca mulatta*, *Proc. Soc. Exper. Biol. & Med.*, **33**:549, 1936.
9. BAITSELL, G. A. On the origin of the connective-tissue ground-substance in the chick embryo, *Quart. J. Micr. Sc.*, **69**:571, 1925.
10. BAKER, B. L., and WHITAKER, W. L. Interference of wound healing by the local action of adrenocortical steroids, *Endocrinology*, **46**:544, 1950.
11. BALDRIDGE, G. D.; KLIGMAN, A. L.; LIPNIK, M. J.; and PILLSBURY, D. M. In vitro effects of cortisone on mesodermal tissue, *Arch. Path.*, **51**:593, 1951.
12. BALÓ, J., and BANGA, I. The elastolytic activity of pancreatic extracts, *Biochem. J.*, **46**:384, 1950.
13. BANGHAM, A. D. The effect of cortisone on wound healing, *Brit. J. Exper. Path.*, **32**:77, 1951.
14. BARBER, A., and NOTHACKER, W. G. Effects of cortisone on healing of non-perforating wounds of cornea in normal and scorbutic guinea pigs, *Federation Proc.*, **11**:408, 1952.
15. BARBER, M., and DELAUNAY, A. Effect of plasma of cortisone-treated guinea-pigs on the growth of fibroblasts and macrophages in tissue cultures in vitro, *J. Path. & Bact.*, **63**:549, 1951.
16. BAUER, H. Mikroskopisch-chemischer Nachweis von Glykogen und einigen anderen Polysacchariden, *Ztschr. f. mikr.-anat. Forsch.*, **33**:143, 1933.
17. BENDITT, E. P., and FRENCH, J. E. Histochemistry of connective tissue. I. The use of enzymes as specific histochemical reagents, *J. Histochem. & Cytochem.*, **1**:315, 1953.
18. BENDITT, E. P.; SCHILLER, S.; MATHEWS, M. B.; and DORFMAN, A. Evidence that hyaluronidase is not the factor in testicular extract causing increased vascular permeability, *Proc. Soc. Exper. Biol. & Med.*, **77**:643, 1951.
19. BENDITT, E. P.; SCHILLER, S.; WONG, H.; and DORFMAN, A. Influence of ACTH and cortisone upon alteration of capillary permeability induced by hyaluronidase in rats, *Proc. Soc. Exper. Biol. & Med.*, **75**:782, 1950.
20. BENSLEY, S. H. On the presence, properties and distribution of the intercellular ground substance of loose connective tissue, *Anat. Rec.*, **60**:93, 1934.
21. BIDWELL, E. Proteolytic enzymes of *Clostridium welchii*, *Biochem. J.*, **46**:589, 1950.
22. BIGNARDI, C. Considerazione critiche sulla reazione di metachromasia, *Atti d. Soc. ital. sc. nat.*, **85**:160, 1946.
23. BLIX, G. Studies in glycoproteins, *Acta physiol. Scandinav.*, **1**:29, 1940.
24. BLIX, G., and SNELLMAN, O. On chondroitin sulphuric acid and hyaluronic acid, *Ark. f. Kem., Min. o. Geol.*, Vol. **19A**, No. 32, 1945; *Chem. Abstr.*, **41**:1260, 1947.
25. BLOOM, F. Effect of cortisone on mast cell tumors (mastocytoma) of the dog, *Proc. Soc. Exper. Biol. & Med.*, **79**:651, 1952.
26. BLUNT, J. W.; PLOTZ, C. M.; LATTES, R.; HOWES, E. L.; MEYER, K.; and RAGAN, C. Effect of cortisone on experimental fractures in the rabbit, *Proc. Soc. Exper. Biol. & Med.*, **73**:678, 1950.
27. BOAS, N. F. Isolation of hyaluronic acid

- from the cock's comb, *J. Biol. Chem.*, **181**:573, 1949.
28. BOAS, N. F., and LUDWIG, A. W. The mechanism of estrogen inhibition of comb growth in the cockerel, with histologic observations, *Endocrinology*, **46**:299, 1950.
29. BOURNE, G. H. Vitamin C and repair of injured tissues, *Lancet*, **2**:661, 1942.
30. BOYLAND, F., and McCLEAN, D. A factor in malignant tissues which increases the permeability of the dermis, *J. Path. & Bact.*, **41**:553, 1935.
31. BREWER, D. B. Myxoedema: an autopsy report with histochemical observations on the nature of mucoid infiltrations, *J. Path. & Bact.*, **63**:503, 1951.
32. BUNTING, H. The distribution of acid mucopolysaccharides in mammalian tissues as revealed by histochemical methods, *Ann. New York Acad. Sc.*, **52**:977, 1950.
33. BURNET, F. M. The mucinase of *V. cholerae*, *Australian J. Exper. Biol. & M. Sc.*, **26**:71, 1948.
34. BUTT, A. J.; HAUSER, E. A.; and SEIFTER, J. Effect of hyaluronidase on urine, and its possible significance in renal lithiasis, *J.A.M.A.*, **150**:1096, 1952.
35. BYCHKOV, S. M. Complexes of hyaluronic acid and chondroitin sulfuric acids with procollagen, *Doklady Akad. Nauk S.S.S.R.*, **75**:83, 1950; *Chem. Abstr.*, **45**:2994, 1951.
36. BYROM, F. B. The nature of myxoedema, *Clin. Sc.*, **1**:273, 1933-34.
37. BYWATERS, E. G. L.; HOLBOROW, E. J.; and KEECH, M. K. Reconstruction of the dermal barrier to dye spread after hyaluronidase injection, *Brit. M. J.*, **2**:1178, 1951.
38. CASTOR, W. C., and BAKER, B. L. The local action of adrenocortical steroids on epidermis and connective tissue of the skin, *Endocrinology*, **47**:234, 1950.
39. CATCHPOLE, H. R. Solubility properties of some components of the ground substance in relation to intravital staining, *Ann. New York Acad. Sc.*, **52**:989, 1950.
40. CAVALLERO, C., and BRACCINI, C. Effect of cortisone on mast cells in the rat, *Proc. Soc. Exper. Biol. & Med.*, **78**:141, 1951.
41. CHAIN, E., and DUTHIE, E. S. A mucolytic enzyme in testis extracts, *Nature*, **144**:977, 1939.
42. ———. Identity of hyaluronidase and spreading factor, *Brit. J. Exper. Path.*, **21**:324, 1940.
43. CLARK, E. R., and CLARK, E. W. Further observations on living lymphatic vessels in the transparent chamber in the rabbit's ear—their relation to the tissue spaces, *Am. J. Anat.*, **52**:273, 1933.
44. ———. Observations on living mammalian lymphatic capillaries. Their relation to blood vessels, *ibid.*, **60**:253, 1937.
45. CLAUDE, A. Spreading property of azo-proteins in the dermis, *J. Exper. Med.*, **62**:229, 1935.
46. ———. Spreading properties of leech extracts and the formation of lymph, *ibid.*, **66**:353, 1937.
47. ———. "Spreading" properties and mucolytic activity of leech extracts, *Proc. Soc. Exper. Biol. & Med.*, **43**:684, 1940.
48. CLAUDE, A., and DURAN-REYNALS, F. On the existence of a factor increasing tissue permeability in organs other than testicle, *J. Exper. Med.*, **60**:457, 1934.
49. CLAYTON, B. E., and PRUNTY, F. T. G. Relation of adrenal cortical function to scurvy in guinea-pigs, *Brit. M. J.*, **2**:927, 1951.
50. COONS, A. H.; LEDUC, E. H.; and KAPLAN, M. H. Localization of antigen in tissue cells, *J. Exper. Med.*, **93**:173, 1951.
51. CREDITOR, M. C.; BEVANS, M.; MUNDY, W. L.; and RAGAN, C. Effect of ACTH on wound healing in humans, *Proc. Soc. Exper. Biol. & Med.*, **74**:245, 1950.
52. CROWLEY, N. Hyaluronidase production by hemolytic streptococci of human origin, *J. Path. & Bact.*, **56**:27, 1944.
53. DANIELLI, J. F.; FELL, H. B.; and KODICEK, E. The enzymes of healing wounds. II. The effect of different degrees of vitamin C-deficiency on the phosphatase activity in experimental wounds in the guinea-pig, *Brit. J. Exper. Path.*, **26**:367, 1945.
54. DAY, T. D. Membranous nature of interstitial connective tissue, *Lancet*, **2**:945, 1947.
55. ———. Hydration of connective tissue (rat fascia), *J. Physiol.*, **109**:380, 1949.
56. ———. The permeability of interstitial connective tissue and the nature of the interfibrillary substance, *ibid.*, **117**:1, 1952.
57. DEMPSEY, E. W. Connective tissues. *In*: Transactions of the First Connective Tissue Conference. New York: Josiah Macy, Jr., Foundation, 1950.

58. DEMPSEY, M., and HAINES, B. M. Nature of the ground substance in interstitial connective tissue, *Nature*, **164**:368, 1949.
59. DIXON, A. S. J., and BYWATERS, E. G. L. The effect of intra-articular injection of cortisone acetate and of hydrocortisone acetate in rheumatoid arthritis, *Clin. Sc.*, **12**:14, 1953.
60. DOCHEZ, A. R., and AVERY, O. T. The elaboration of specific soluble substance by pneumococcus during growth, *J. Exper. Med.*, **26**:477, 1917.
61. DODDS, E. C., and ROBERTSON, J. D. The clinical application of dinitro-*o*-cresol. II. A study of myxoedema, *Lancet*, **2**:1197, 1933.
62. DORFMAN, A. The *in vitro* action of hyaluronidase, *Ann. New York Acad. Sc.*, **52**:1017, 1950.
63. ———. The action of serum on hyaluronidase, *ibid.*, p. 1098.
64. ———. The effects of adrenal hormones on connective tissue, *ibid.*, **56**:698, 1953.
65. DORFMAN, A., and OTT, M. L. A turbidimetric method for the assay of hyaluronidase, *J. Biol. Chem.*, **172**:367, 1948.
66. DOUGHERTY, F. Topical antiphlogistic action of cortisone, *Federation Proc.*, **10**:36, 1951.
67. DRENNAN, J. M. The mast cells in urticaria pigmentosa, *J. Path. & Bact.*, **63**:513, 1951.
68. DURAN-REYNALS, F. The effect of extracts of certain organs from normal and immunized animals on the infecting power of vaccine virus, *J. Exper. Med.*, **50**:327, 1929.
69. ———. The effect of testicle extract and of normal serum on a transplantable epithelial tumor of the rabbit, *ibid.*, **54**:493, 1931.
70. ———. Studies on a certain spreading factor existing in bacteria and its significance for bacterial invasiveness, *ibid.*, **58**:161, 1933.
71. ———. A spreading factor in certain snake venoms and its relation to their mode of action, *ibid.*, **69**:69, 1939.
72. ———. Tissue permeability and the spreading factors in infection, *Bact. Rev.*, **6**:197, 1942.
73. DURAN-REYNALS, F.; BUNTING, H.; and VAN WAGENEN, G. Studies on the sex skin of *Macaca mulatta*, *Ann. New York Acad. Sc.*, **52**:1006, 1950.
74. EBERT, R. H. Personal communication.
75. EBERT, R. H., and WISSLER, R. W. In vivo observations of the effects of cortisone on the vascular reaction to large doses of horse serum using the rabbit ear chamber technique, *J. Lab. & Clin. Med.*, **38**:497, 1951.
76. EHRLICH, W. E. Nature of collagen diseases, *Am. Heart. J.*, **43**:121, 1952.
77. EHRLICH, W. E.; SEIFTER, J.; ALBURN, H. E.; and BEGANY, A. J. Heparin and heparinocytes in elephantiasis scroti, *Proc. Soc. Exper. Biol. & Med.*, **70**:183, 1949.
78. EINBINDER, J., and SCHUBERT, M. Binding of mucopolysaccharides and dyes by collagen, *J. Biol. Chem.*, **188**:335, 1951.
79. ELSTER, S. K. Effect of ascorbic acid deficiency on the collagen content of guinea pig tissues, *Am. J. Path.*, **26**:711, 1950.
80. ELSTER, S. K.; FREEMAN, M. E.; and DORFMAN, A. Effect of hyaluronidase on the passage of fluid and of T-1824 through the capillary wall, *Am. J. Physiol.*, **156**:429, 1949.
81. ENGEL, M. B. Mobilization of mucoprotein by parathyroid extract, *Arch. Path.*, **53**:339, 1952.
82. FELL, H. B., and DANIELLI, J. F. The enzymes of healing wounds. I. The distribution of alkaline phosphomonoesterase in experimental wounds and burns in the rat, *Brit. J. Exper. Path.*, **24**:196, 1943.
83. FISCHER, E. E. Hypersensitivity and the hyperadrenal state. *In*: Transactions of the Third Connective Tissue Conference. New York: Josiah Macy, Jr., Foundation, 1952.
84. FISCHER, A. The participation of blood proteins in the metabolism of tissue cells, *Biol. Rev.*, **22**:178, 1947.
85. FLEMMING, W. Beiträge zur Anatomie und Physiologie des Bindegewebes, *Arch. f. mikr. Anat.*, **12**:391, 1876.
86. ———. Zur Entwicklungsgeschichte der Bindegewebsfibrillen, *Internat. Beitr. z. wissensch. med. Festschr. f. Virchow*, Berlin, **1**:213, 1891.
87. FOLLIS, R. H., JR. Effect of proteolytic enzymes and fixation on metachromasia of skin collagen, *Proc. Soc. Exper. Biol. & Med.*, **76**:272, 1951.
88. FREEMAN, M. E., and WEBSTER, M. E. Stability of purified hyaluronidase, *Proc. Soc. Exper. Biol. & Med.*, **79**:113, 1952.
89. FRENCH, J. E., and BENDITT, E. P. The

- histochemistry of connective tissue. II. The effect of proteins on the selective staining of mucopolysaccharides by basic dyes, *J. Histochem. & Cytochem.*, **1**:321, 1953.
90. ———. Observations on the localization of alkaline phosphatase in healing wounds. *Arch. Path.* (in press).
91. FRIOU, G. J. Further observations of an inhibitor in human serums of the hyaluronidase produced by a strain of hemolytic streptococcus, *Ann. New York Acad. Sc.*, **52**:1112, 1950.
92. FRUTON, J. S. The role of proteolytic enzymes in the biosynthesis of peptide bonds, *Yale J. Biol. & Med.*, **22**:263, 1950.
93. FULTON, J. K.; STANLEY, M.; and ROBINSON, W. D. Use of the streptococcal decapsulation test as a measure of thermolabile hyaluronidase inhibitor in sera, *Ann. New York Acad. Sc.*, **52**:1133, 1950.
94. GERSH, I. Ground substance and the plasticity of connective tissues, *Harvey Lect.*, p. 211, 1949–50.
95. ———. Some fundamental considerations of ground substance of connective tissues. *In*: Transactions of the Second Connective Tissue Conference. New York: Josiah Macy, Jr., Foundation, 1951.
96. GERSH, I., and CATCHPOLE, H. R. The organization of ground substance and basement membrane and its significance in tissue injury, disease and growth, *Am. J. Anat.*, **85**:457, 1949.
97. GLICK, D. Hyaluronidase inhibitor of human blood serum in health and disease, *J. Mt. Sinai Hosp.*, **17**:207, 1950–51.
98. GLICK, D., and GRAIS, M. L. Concerning the alleged occurrence of hyaluronidase in skin (letter to the editor), *Arch. Biochem.*, **18**:511, 1948.
99. GLICK, D.; KIRILUK, L. B.; HAKANSEN, E. Y.; and KREMEN, A. J. Question of hyaluronidase in tumor tissue, *Federation Proc.*, **9**:177, 1950.
100. GLICK, D., and OCHS, M. J. Mucolytic enzyme systems. XIX. Comparison of hyaluronidase inhibitor and heparin levels in serum, *Proc. Soc. Exper. Biol. & Med.*, **81**:363, 1952.
101. GLYNN, L. E., and HOLBOROW, E. J. Conversion of tissue polysaccharides to auto-antigens by group-A beta-hemolytic streptococci, *Lancet*, **2**:449, 1952.
102. GLYNN, L. E., and LOEWI, G. Fibrinoid necrosis in rheumatic fever, *J. Path. & Bact.*, **64**:329, 1952.
103. GLYNN, L. E.; LOEWI, G.; and CONSDEN, R. Investigation of fibrinoid, *Lancet*, **2**:166, 1951.
104. GOMORI, G. Aldehyde-fuchsin—a new stain for elastic tissue, *Am. J. Clin. Path.*, **20**:665, 1950.
105. ———. Microscopic histochemistry: principles and practice. Chicago: University of Chicago Press, 1952.
106. GROSS, J. Electron microscope studies of sodium hyaluronate, *J. Biol. Chem.*, **172**:511, 1948.
107. ———. A study of certain connective tissue constituents with the electron microscope, *Ann. New York Acad. Sc.*, **52**:964, 1950.
108. ———. A study of the aging of collagenous connective tissue of rat skin with the electron microscope, *Am. J. Path.*, **26**:708, 1950.
109. GROSS, J.; HIGHBERGER, J. H.; and SCHMITT, F. O. Some factors involved in the fibrogenesis of collagen in vitro, *Proc. Soc. Exper. Biol. & Med.*, **80**:462, 1952.
110. GUNTER, G. S. The determination of "Spinnbarkeit" of synovial fluid and its destruction by enzymatic action, *Australian J. Exper. Biol. & M. Sc.*, **27**:265, 1949.
111. HAAS, E. On the mechanism of invasion. I. Anti-invasin I, an enzyme of plasma, *J. Biol. Chem.*, **163**:63, 1946.
112. HAHN, L. On the mucopolysaccharide-splitting enzyme system of the mammalian testis, *Ark. f. Kem., Min. o. Geol.*, Vol. **21A**, No. 1, 1946.
113. HALE, C. W. Histochemical demonstration of acid polysaccharides in animal tissues, *Nature*, **157**:802, 1946.
114. HALL, D. A.; REED, R.; and TUNBRIDGE, R. E. Structure of elastic tissue, *Nature*, **170**:264, 1952.
115. HALLIBURTON, W. D. Report of chemical investigations of the tissues and organs from cases of myxoedema in men and animals. *In*: Report of committee on myxoedema, *Tr. Clin. Soc., Suppl. to Vol. 21*, Clin. Investigation, pp. 48–60, 1888.
116. HARDESTY, M. The structural basis for the response of the comb of the brown Leghorn fowl to sex hormones, *Am. J. Anat.*, **47**:277, 1931.
117. HASS, G. M. Studies of amyloid. 2. The

- isolation of a polysaccharide from amyloid-bearing tissues, *Arch. Path.*, **34**:92, 1942.
118. HASS, G. M.; HUNTINGTON, R.; and KRUMDIECK, N. Amyloid. 3. The properties of amyloid deposits occurring in several species under diverse conditions, *Arch. Path.*, **35**:226, 1943.
 119. HASS, G. M., and McDONALD, F. Studies of collagen. I. The production of collagen in vitro under variable experimental conditions, *Am. J. Path.*, **16**:525, 1940.
 120. HAYES, M. A., and REED, T. G. Factors influencing measurement of hyaluronidase activity by dermal spread of an indicator, *Proc. Soc. Exper. Biol. & Med.*, **75**:357, 1950.
 121. HECHTER, O. Mechanism of hyaluronidase action in skin, *Science*, **104**:409, 1946.
 122. ———. Studies on spreading factors. I. Importance of mechanical factors in hyaluronidase action in skin, *J. Exper. Med.*, **85**:77, 1947.
 123. ———. Mechanisms of spreading factor action, *Ann. New York Acad. Sc.*, **52**:1028, 1950.
 124. HEHRE, E. J. Enzymic synthesis of polysaccharides: a biological type of polymerization. *In: Advances in enzymology*, **9**:297. New York: Interscience Publishers, Inc., 1951.
 125. HEIDELBERGER, M. The chemical nature of immune substances, *Physiol. Rev.*, **7**:107, 1927.
 126. HEIDELBERGER, M.; GOEBEL, W. F.; and AVERY, O. T. The soluble specific substance of pneumococci, *J. Exper. Med.*, **42**:727, 1925.
 127. HEILMEYER, L., and BEGEMANN, H. Hautkrankheiten bei Dysproteinämien, *Hautarzt*, **1**:59, 1950.
 128. HIGHBERGER, J. H.; GROSS, J.; and SCHMITT, F. O. Electron microscope observations of certain fibrous structures obtained from connective tissue extracts, *J. Am. Chem. Soc.*, **72**:3321, 1950.
 129. ———. The interaction of mucoprotein with soluble collagen; an electron microscope study, *Proc. Nat. Acad. Sc.*, **37**:286, 1951.
 130. HOLBOROW, E. J., and KEECH, M. K. Hyaluronidase skin spreading effect, *Brit. M. J.*, **2**:1178, 1951.
 131. HOTCHKISS, R. D. A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations, *Arch. Biochem.*, **16**:131, 1948.
 132. HUMPHREY, J. H. Studies on diffusing factors. New biological assay of diffusing factors in guinea pigs, *Biochem. J.*, **37**:177, 1943.
 133. ———. Antigenic properties of hyaluronic acid, *ibid.*, p. 460.
 134. ———. Studies on diffusing factors. 1. The kinetics of the action of hyaluronidase from various sources upon hyaluronic acid, with a note upon anomalies encountered in the estimation of N-acetyl glucosamine, *ibid.*, **40**:435, 1946.
 135. ———. Studies on diffusing factors. 2. The action of hyaluronidase preparations from various sources upon some substrates other than hyaluronic acid, *ibid.*, p. 442.
 136. JANES, J., and MACDONALD, J. R. Mast cells. Their distribution in various human tissues, *Arch. Path.*, **45**:622, 1948.
 137. JENEY, A. VON, and TÖRÖ, E. Die Wirkung der Ascorbinsäure auf die Faserbildung in Fibroblastkulturen, *Virchows Arch. f. path. Anat.*, **298**:87, 1936–37.
 138. JONES, I. S., and MEYER, K. Inhibition of vascularization of the rabbit cornea by local application of cortisone, *Proc. Soc. Exper. Biol. & Med.*, **74**:102, 1950.
 139. JORPES, J. E. Heparin. London: Oxford University Press, 1946.
 140. JORPES, J. E., and BERGSTROM, S. Heparin: a mucoitin polysulfuric acid, *J. Biol. Chem.*, **118**:447, 1937.
 141. JORPES, J. E., and GARDELL, S. On heparin monosulfuric acid, *J. Biol. Chem.*, **176**:267, 1948.
 142. JORPES, J. E.; WERNER, B.; and ABERG, B. The fuchsin-sulfurous acid test after periodate on heparin and allied polysaccharides, *J. Biol. Chem.*, **176**:277, 1948.
 143. KASS, E. H., and SEASTONE, C. V. The role of the mucoid polysaccharide (hyaluronic acid) in the virulence of group A hemolytic streptococci, *J. Exper. Med.*, **79**:319, 1944.
 144. KENDALL, F. E.; HEIDELBERGER, M.; and DAWSON, M. H. A serologically inactive polysaccharide elaborated by mucoid strains of group A hemolytic streptococcus, *J. Biol. Chem.*, **118**:61, 1937.
 145. KLEMPERER, P. The concept of collagen disease, *Am. J. Path.*, **26**:505, 1950.
 146. KLEMPERER, P.; POLLACK, A. D.; and

- BAEHR, G. Diffuse collagen disease, *J.A.M.A.*, **119**:331, 1942.
147. KNELLER, M. H., and WELLS, G. C. Unpublished experiments, 1953.
 148. KÖLLIKER, A. Neue Untersuchungen über die Entwicklung des Bindegewebes, *Verhandl. d. phys.-med. Gesellsch.*, **2**:141, 1861.
 149. LANDIS, E. M. Capillary permeability and the factors affecting the composition of capillary filtrate, *Ann. New York Acad. Sc.*, **46**:713, 1945-46.
 150. LANDSTEINER, K. The specificity of serological reactions. Artificial conjugated antigens, pp. 156-207. Cambridge: Harvard University Press, 1945.
 151. LANDSTEINER, K., and LEVINE, P. On the Forssman antigens in *B. paratyphosus* B and *B. dysenteriae* Shiga, *J. Immunol.*, **22**:75, 1932.
 152. LAYTON, L. L. In vitro sulfate fixation by granulation tissue and injured muscle tissue from healing wounds, *Proc. Soc. Exper. Biol. & Med.*, **73**:570, 1950.
 153. ———. The anabolic metabolism of radioactive sulfate by animal tissues in vitro and in vivo, *Cancer*, **4**:198, 1951.
 154. ———. Effect of cortisone upon chondroitin sulfate synthesis by animal tissues, *Proc. Soc. Exper. Biol. & Med.*, **76**:596, 1951.
 155. ———. Cortisone inhibition of mucopolysaccharide synthesis in the intact rat (letter), *Arch. Biochem.*, **32**:224, 1951.
 156. LETTERER, E. Neue Untersuchungen über die Entstehung des Amyloids, *Virchows Arch. f. path. Anat.*, **293**:34, 1934.
 157. LEVENE, P. A. Hexosamine and mucoproteins. London and New York: Longmans, Green & Co., 1925.
 158. LEVINE, A., and SCHUBERT, M. Metachromasy of thiazine dyes produced by chondroitin sulfate, *J. Am. Chem. Soc.*, **74**:91, 1952.
 159. LEVINE, M. D.; GARZOLI, R. F.; KUNTZ, R. E.; and KILLOUGH, J. H. On the demonstration of hyaluronidase in cercaria of *Schistosoma mansoni*, *J. Parasitol.*, **34**:158, 1948.
 160. LEVINE, R.; GOLDSTEIN, M. S.; RAMEY, E. R.; and FRITZ, I. Studies on the mode of action of cortisone in stress situations, *Bull. New England M. Center*, **13**:114, 1951.
 161. LILLIE, R. D. Connective tissue staining. In: Transactions of the Third Connective Tissue Conference. New York: Josiah Macy, Jr., Foundation, 1952.
 162. LILLIE, R. D.; EMMART, E. W.; and LASKEY, A. M. Chondromucinas from bovine testis and the chondromucin of umbilical cord, *Arch. Path.*, **52**:363, 1951.
 163. LUDWIG, A. W., and BOAS, N. F. The effect of testosterone on the connective tissue of the comb of the cockerel, *Endocrinology*, **46**:291, 1950.
 164. LUDWIG, A. W.; BOAS, N. F.; and SOFFER, L. J. Role of mucopolysaccharides in pathogenesis of experimental exophthalmos, *Proc. Soc. Exper. Biol. & Med.*, **73**:137, 1950.
 165. LURIE, M. B. Mechanism affecting spread in tuberculosis, *Ann. New York Acad. Sc.*, **52**:1074, 1950.
 166. LURIE, M. B., and ZAPPASODI, P. Effect of gonadotropin on the spread of particulate substances in the skin of rabbits, *Arch. Path.*, **34**:151, 1942.
 167. MADINAVEITIA, J., and QUIBELL, T. H. H. Studies on diffusing factors. VI. The action of testicular extracts on the viscosity of vitreous humor preparations, *Biochem. J.*, **34**:625, 1940.
 168. ———. Studies on diffusing factors. VIII. The reduction of the viscosity of vitreous humor preparations by ascorbic acid and some diazo compounds, *ibid.*, **35**:453, 1941.
 169. MANCINI, R. E., and SACERDOTE DE LUSTIG, E. Histochemical study on the action of hyaluronidase upon fibroblasts cultivated in vitro, *J. Nat. Cancer Inst.*, **10**:1371, 1950.
 170. MASCHMANN, E. Über Backterienproteasen. IX. Die Anaerobiase, *Biochem. Ztschr.*, **297**:284, 1938.
 171. MATTHEWS, B. F. Collagen/chondroitin sulphate ratio of human articular cartilage related to function, *Lancet*, **2**:1295, 1952.
 172. MATHEWS, M. B., and DORFMAN, A. The molecular weight and viscosity of chondroitin sulphuric acid, *Arch. Biochem.*, **42**:41, 1952.
 173. MATHEWS, M. B.; ROSEMAN, S.; and DORFMAN, A. Determination of the chondrosamine activity of bovine testicular preparations, *J. Biol. Chem.*, **188**:327, 1951.
 174. MAXIMOW, A. A. Development of argyrophile and collagenous fibers in tissue cul-

- tures, Proc. Soc. Exper. Biol. & Med., **25**: 439, 1927.
175. MAXIMOW, A. A., and BLOOM, W. A text-book of histology. 6th ed. Philadelphia and London: W. B. Saunders Co., 1952.
 176. MAYER, R. L. Hyaluronidase and inflammation of the skin, Ann. New York Acad. Sc., **52**:1041, 1950.
 177. MENKIN, V. Effect of adrenal cortex extract on capillary permeability, Am. J. Physiol., **129**:691, 1940.
 178. MEYER, K. Mucoids and glycoproteins. In: Advances in protein chemistry, **2**:249. New York: Interscience Publishers, Inc., 1945.
 179. ———. The biological significance of hyaluronic acid and hyaluronidase, Physiol. Rev., **27**:335, 1947.
 180. ———. Cement substances of connective tissue, Ann. Rheumat. Dis., **7**:33, 1948.
 181. ———. The action of hyaluronidases on hyaluronic acid, Ann. New York Acad. Sc., **52**:1021, 1950.
 182. ———. Connective tissues. In: Transactions of the First Connective Tissue Conference. New York: Josiah Macy, Jr., Foundation, 1950.
 183. ———. Physical chemistry of proteins, Nature, **170**:518, 1952.
 184. MEYER, K., and CHAFFEE, E. The mucopolysaccharides of the skin, J. Biol. Chem., **138**:491, 1941.
 185. MEYER, K.; CHAFFEE, E.; HOBBY, G. L.; and DAWSON, M. H. Hyaluronidases of bacterial and animal origin, J. Exper. Med., **73**:309, 1941.
 186. MEYER, K.; DUBOS, R.; and SMITH, E. M. The hydrolysis of polysaccharide acids of vitreous humor, of umbilical cord and of *Streptococcus* by the autolytic enzymes of *Pneumococcus*, J. Biol. Chem., **118**:71, 1937.
 187. MEYER, K., and PALMER, J. W. The polysaccharide of the vitreous humor, J. Biol. Chem., **107**:629, 1934.
 188. ———. On glycoproteins. II. The polysaccharides of vitreous humor and of umbilical cord, *ibid.*, **114**:689, 1936.
 189. MEYER, K., and RAPPORT, M. M. The mucopolysaccharides of the ground substance of connective tissue, Science, **113**:596, 1951.
 190. ———. Hyaluronidases. In: Advances in enzymology, **13**:199. New York: Interscience Publishers, Inc., 1952.
 191. MEYER, K.; SMYTH, E. M.; and DAWSON, M. H. The isolation of a mucopolysaccharide from synovial fluid, J. Biol. Chem., **128**:319, 1939.
 192. MICHAEL, M., and WHORTON, C. M. Delay of the early inflammatory response by cortisone, Proc. Soc. Exper. Biol. & Med., **76**:754, 1951.
 193. MICHAELIS, L., and GRANICK, S. Metachromasy of basic dyestuffs, J. Am. Chem. Soc., **67**:1212, 1945.
 194. MONTAGNA, W.; CHASE, H. B.; and MELARAGNO, H. P. Histology and cytochemistry of human skin. I. Metachromasia in the mons pubis, J. Nat. Cancer Inst., **12**:591, 1951.
 195. MOON, V. H., and TERSHAKOVEC, G. A. Influence of cortisone upon acute inflammation, Proc. Soc. Exper. Biol. & Med., **79**:63, 1952.
 196. MORGAN, W. T. J., and PARTRIDGE, S. M. Studies in immunochemistry. 4. The fractionation and nature of antigenic material isolated from *Bact. dysenteriae* (Shiga), Biochem. J., **34**:169, 1940.
 197. MORRIONE, T. G. The formation of collagen fibers by the action of heparin on soluble collagen: an electron microscope study, J. Exper. Med., **96**:107, 1952.
 198. MOSBACH, E. H., and KING, C. G. Tracer studies on the biosynthesis of glucuronic acid, Federation Proc., **9**:208, 1950.
 199. MURRAY, R. G. E., and PEARCE, R. H. The detection and assay of hyaluronidase by means of mucoid streptococci, Canad. J. Research (sec. E), **27**:254, 1949.
 200. MCCLEAN, D. The influence of testicular extract on dermal permeability and response to vaccine virus, J. Path. & Bact., **33**:1045, 1930.
 201. ———. A factor in culture filtrates of certain pathogenic bacteria which increases the permeability of the tissues, *ibid.*, **42**:477, 1936.
 202. ———. The capsulation of streptococci and its relation to diffusion factor (hyaluronidase), *ibid.*, **53**:13, 1941.
 203. ———. The in-vivo decapsulation of streptococci by hyaluronidase, *ibid.*, **54**:284, 1942.
 204. ———. Studies on diffusing factors. 2. Methods of assay of hyaluronidase and their correlation with skin diffusing activity, Biochem. J., **37**:169, 1943.
 205. MCCLEAN, D., and HALE, C. W. Studies on diffusing factors. The hyaluronidase

- activity of testicular extracts, bacterial culture filtrates and other agents that increase tissue permeability, *Biochem. J.*, **35**:159, 1941.
206. McCLEAN, D.; ROGERS, H. J.; and WILLIAMS, B. W. Early diagnosis of wound infection with special reference to gas gangrene, *Lancet*, **1**:355, 1943.
207. McCQUIRE, W. B., and ALDRICH, C. A. Time required for disappearance of intradermally injected salt solution, *J.A.M.A.*, **81**:293, 1923.
208. MACFARLANE, R. G., and MACLENNAN, J. D. The toxæmia of gas-gangrene, *Lancet*, **2**:328, 1945.
209. McMANUS, J. F. A. Histological demonstration of mucin after periodic acid, *Nature*, **158**:202, 1946.
210. ———. Carbohydrate specificity of the periodic acid-Schiff's reagent (P.A.S.) method, *Am. J. Path.*, **26**:690, 1950.
211. ———. Techniques for histochemical approach to pathology. *In*: Progress in fundamental medicine, p. 151. Philadelphia: Lea & Febiger, 1952.
212. McMANUS, J. F. A., and CASON, J. E. Carbohydrate histochemistry studied by acetylation techniques. I. Periodic acid methods, *J. Nat. Cancer Inst.*, **10**:1343, 1950.
213. McMASTER, P. D., and PARSONS, R. J. The movement of substances and the state of the fluid in the intradermal tissue, *Ann. New York Acad. Sc.*, **52**:992, 1950.
214. NAGEOTTE, J., and GUYON, L. Considérations générales sur la trame conjonctive, *Arch. de biol.*, Paris, **41**:1, 1930.
215. OAKLEY, C. L., and WARRACK, G. H. The acra test as a means of estimating hyaluronidase, desoxyribonuclease and their antibodies, *J. Path. & Bact.*, **63**:45, 1951.
216. OAKLEY, C. L.; WARRACK, G. H.; and VAN HEYNINGEN, W. E. The collagenase (kappa-toxin) of *Cl. welchii* type A, *J. Path. & Bact.*, **58**:229, 1946.
217. OGSTON, A. G.; PHILPOT, J. ST. L.; and ZUCKERMAN, S. Observations related to the swelling of sexual skin in rhesus monkeys, *J. Endocrinol.*, **1**:231, 1939.
218. OGSTON, A. G., and STANIER, J. E. On the state of hyaluronic acid in synovial fluid, *Biochem. J.*, **46**:364, 1950.
219. OLIVER, J.; BLOOM, F.; and MANGIERI, C. On the origin of heparin. An examination of the heparin content and the specific cytoplasmic particles of neoplastic mast cells, *J. Exper. Med.*, **86**:107, 1947.
220. OPSAHL, J. C. Elevated environmental temperature: its possible influence on the action of spreading factor, *Yale J. Biol. & Med.*, **21**:433, 1948-49.
221. ———. The role of certain steroids in the adrenal-hyaluronidase relationship, *Yale J. Biol. & Med.*, **22**:115, 1949.
222. ORD, W. M. On myxoedema, a term proposed to be applied to an essential condition in the "cretinoid" affection occasionally observed in middle-aged women, *M. Chir. Tr.* **61**:57, 1878.
223. OREKHOVICH, K. D. The procollagen content of skin in animals of different ages, *Doklady Akad. Nauk S.S.S.R.*, **71**:521, 1950; *Chem. Abstr.*, **44**:8453, 1950.
224. OREKHOVICH, V. N.; TUSTANOVSKII, A. A.; OREKHOVICH, K. D.; and PLOTNIKOV, N. E. The procollagen of hide, *Biokhimiya*, **13**:55, 1948; *Chem. Abstr.*, **42**:7805, 1948.
225. PAFF, G. H.; BLOOM, F. H.; and REILLY, C. The morphology and behaviour of neoplastic mast cells cultivated in vitro, *J. Exper. Med.*, **86**:117, 1947.
226. PEARCE, R. H., and WATSON, E. M. The mucopolysaccharides of human skin, *Canad. J. Research (sec. E)*, **27**:43, 1949.
227. PENNEY, J. R., and BALFOUR, B. M. The effect of vitamin C on mucopolysaccharide production in wound healing, *J. Path. & Bact.*, **61**:171, 1949.
228. PERCIVAL, G. H.; HANNAY, P. W.; and DUTHIE, D. A. Fibrous changes in the dermis, with special reference to senile elastosis, *Brit. J. Dermat.*, **61**:269, 1949.
229. PERL, E., and CATCHPOLE, H. R. Changes induced in the connective tissues of the pubic symphysis of the guinea pig with estrogen and relaxin, *Arch. Path.*, **50**:233, 1950.
230. PIGMAN, W. W., and GOEPP, R. M. Chemistry of the carbohydrates. New York: Academic Press, Inc., 1948.
231. PIKE, R. M. The production of hyaluronic acid and hyaluronidase by some strains of group A streptococci, *Ann. New York Acad. Sc.*, **52**:1071, 1950.
232. PIRANI, C. L. Collagen diseases: Paper read at the International Congress of Clinical Pathology, *Lancet*, **2**:166, 1951.
233. PIRANI, C. L., and CATCHPOLE, H. R. Serum glycoproteins in experimental scurvy, *Arch. Path.*, **51**:597, 1951.

234. PIRANI, C. L., and STEPTO, R. C. Tissue changes induced by desoxycorticosterone acetate (DCA) in the guinea pig, with particular regard to connective tissue, *Am. J. Path.*, **26**:749, 1950.
235. PLOTZ, C. M.; HOWES, E. L.; MEYER, K.; BLUNT, J. W.; LATTES, R.; and RAGAN, C. The effect of the hyperadrenal state on connective tissue, *Am. J. Path.*, **26**:709, 1950.
236. PORGES, N. Snake venoms, their biochemistry and mode of action, *Science*, **117**:47, 1953.
237. PORTER, K. R. Repair processes in connective tissues. *In*: Transactions of the Second Connective Tissue Conference. New York: Josiah Macy, Jr., Foundation, 1951.
238. PRAKKEN, J. R., and WOERDEMAN, M. J. Mast cells in diseases of the skin; their relation to tissue eosinophilia, *Dermatologica*, **105**:116, 1952.
239. PROSE, P. H., and BAER, R. L. Assay of hyaluronidase in various dermatoses, *J. Invest. Dermat.*, **16**:169, 1951.
240. QUINN, R. W. The antihyaluronidase content of blood serum. A study of sera from patients with rheumatic fever, streptococcal infection, miscellaneous non-streptococcal diseases, and from normal individuals of different ages, *Ann. New York Acad. Sc.*, **52**:1118, 1950.
241. RAGAN, C. Ascorbic acid metabolism, *Proc. Second Clin. ACTH Conference*, **1**:366, 1951.
242. RAGAN, C.; HOWES, E. L.; PLOTZ, C. M.; MEYER, K.; and BLUNT, J. W. Effect of cortisone on production of granulation tissue in the rabbit, *Proc. Soc. Exper. Biol. & Med.*, **72**:718, 1949.
243. RAGAN, C., and MEYER, K. The hyaluronic acid of synovial fluid in rheumatoid arthritis, *J. Clin. Investigation*, **28**:56, 1949.
244. REPPERT, E.; DONEGAN, J.; and HINES, L. E. Ascorbic acid and the hyaluronidase hyaluronic acid reaction, *Proc. Soc. Exper. Biol. & Med.*, **77**:318, 1951.
245. RITTER, H. B., and OLESON, J. J. Combined histochemical staining of acid polysaccharides and 1,2-glycol groupings in paraffin sections of rat tissues, *Am. J. Path.*, **26**:639, 1950.
246. ROBB-SMITH, A. H. T. Tissue changes induced by *Cl. welchii* type A filtrates, *Lancet*, **2**:362, 1945.
247. ———. The nature of reticulin. *In*: Transactions of the Third Connective Tissue Conference. New York: Josiah Macy, Jr., Foundation, 1952.
248. ———. Discussion of the collagen vascular diseases, *Proc. Roy. Soc. Med.*, **45**:811, 1952.
249. ROBERTSON, W. B.; ROPES, M. W.; and BAUER, W. Mucinase: a bacterial enzyme which hydrolyses synovial fluid mucin and other mucins, *J. Biol. Chem.*, **133**:261, 1940.
250. ROBSON, H. N., and DUTHIE, J. J. R. Capillary resistance and adreno-cortical activity, *Brit. M. J.*, **2**:971, 1950.
251. ———. Further observations on capillary resistance and adrenocortical activity, *ibid.*, **1**:994, 1952.
252. ROGERS, H. J. The influence of hydrolysates of hyaluronate upon hyaluronidase production by micro-organisms, *Biochem. J.*, **40**:583, 1946.
253. ROLLHÄUSER, H. Experimentelle Beeinflussung der Dehnbarkeit des Bindegewebes am lebenden Organismus, *Klin. Wchnschr.*, **26**:126, 1948.
254. ROPES, M. W.; ROBERTSON, W. V. B.; ROSSMEISL, E.; PEABODY, R. B.; and BAUER, W. Synovial fluid mucin, *Acta med. Scandinav. (suppl.)*, **196**:700, 1947.
255. ROSEMAN, S.; LUDOWIEG, J.; MOSES, F.; and DORFMAN, A. The biosynthesis of glucuronic acid moiety of hyaluronic acid, *Arch. Biochem.*, **42**:472, 1953.
256. ROSEMAN, S.; MOSES, F. E.; LUDOWIEG, J.; and DORFMAN, A. Biosynthesis of hyaluronic acid by hemolytic streptococci, *Federation Proc.*, **11**:276, 1952.
257. ———. The biosynthesis of hyaluronic acid by group A streptococcus. I. Utilization of 1-C¹⁴-glucose, *J. Biol. Chem.*, **203**:213, 1953.
258. SALLMAN, B., and BIRKELAND, J. M. The role of hyaluronidase in hemolytic streptococcal infection, *Ann. New York Acad. Sc.*, **52**:1062, 1950.
259. SCHILLER, S.; BENDITT, E. P.; and DORFMAN, A. Effect of testosterone and cortisone on the hexosamine content and metachromasia of chick combs, *Endocrinology*, **50**:504, 1952.
260. SEEBERG, G. Cutaneous absorption during the menstrual cycle and its influence on intradermal reactions of the delayed type, *Acta dermat.-venereol.*, **30**:231, 1950.

261. SEIFTER, J. Studies on the pharmacology and toxicology of testicular hyaluronidase, *Ann. New York Acad. Sc.*, **52**:1141, 1950.
262. SEIFTER, J.; BAEDER, D. H.; and BEGANY, A. J. Influence of hyaluronidase and steroids on permeability of synovial membrane, *Proc. Soc. Exper. Biol. & Med.*, **72**:277, 1949.
263. SEIFTER, J.; BAEDER, D. H.; and DERVINIS, A. Alteration in permeability of some membranes by hyaluronidase and inhibition of this effect by steroids, *Proc. Soc. Exper. Biol. & Med.*, **72**:136, 1949.
264. SEVILLE, R. H. Scleroderma-dermatomyositis (with electromicrographs), *Brit. J. Dermat.*, **64**:467, 1952.
265. SHAPIRO, R.; TAYLOR, B.; and TAUBENHAUS, M. Local effects of cortisone on granulation tissue and the role of denervation and ischemia, *Proc. Soc. Exper. Biol. & Med.*, **76**:854, 1951.
266. SIMPSON, W. L. Mucolytic enzymes and invasion by carcinomas, *Ann. New York Acad. Sc.*, **52**:1125, 1950.
267. SPAIN, D. M., and MOLOMUT, N. Effect of cortisone on inflammation in mice, *Am. J. Clin. Path.*, **22**:944, 1952.
268. SPAIN, D. M.; MOLOMUT, N.; and HABER, A. The effect of cortisone on the formation of granulation tissue in mice, *Am. J. Path.*, **26**:710, 1950.
269. SPECTOR, W. G. The role of some higher peptides in inflammation, *J. Path. & Bact.*, **63**:93, 1951.
270. SPRUNT, D. H. The ground substance in infection, *Ann. New York Acad. Sc.*, **52**:1052, 1950.
271. STACEY, M. The chemistry of mucopolysaccharides and mucoproteins. *In: Advances in carbohydrate chemistry*, **2**:161. New York: Interscience Publishers, Inc., 1946.
272. STEARNS, M. L. Studies on the development of connective tissue in transparent chambers in the rabbit's ear, *Am. J. Anat.*, **66**:133, 1940.
273. ———. Studies on the development of connective tissue in transparent chambers in the rabbit's ear. II, *ibid.*, **67**:55, 1940.
274. STEWART, C. T.; SALMON, R. J.; and MAY, C. D. Function of the adrenal cortex in ascorbic acid deficiency and scurvy in the monkey, *J. Lab. & Clin. Med.*, **40**:657, 1952.
275. STOUGHTON, R. B., and LORINCZ, A. L. The action of collagenase on skin and the anticollagenase factor in human serum, *J. Invest. Dermat.*, **16**:43, 1951.
276. STOUGHTON, R. B., and WELLS, G. C. A histochemical study of polysaccharides in normal and diseased skin, *J. Invest. Dermat.*, **14**:37, 1950.
277. SYLVÉN, B. Über das Vorkommen von hochmolekularen Esterschwefelsäuren im Granulationsgewebe und bei Epithelregeneration, *Acta chir. Scandinav.*, Vol. **86**, suppl. 66, 1941.
278. ———. The qualitative distribution of metachromatic polysaccharide material during hair growth, *Exper. Cell Research*, **1**:582, 1950.
279. ———. Mast cells. Cytoplasmic constituents, *ibid.*, **2**:252, 1951.
280. SYLVÉN, B., and MALMGREN, H. On the alleged metachromasia of hyaluronic acid, *Lab. Investigation*, **1**:413, 1952.
281. TAUBENHAUS, M., and AMROMIN, G. D. Influence of steroid hormones on granulation tissue, *Endocrinology*, **44**:359, 1949.
282. ———. The effects of the hypophysis, thyroid, sex steroids and adrenal cortex upon granulation tissue, *J. Lab. & Clin. Med.*, **36**:7, 1950.
283. TAUBENHAUS, M.; TAYLOR, B.; and MORTON, J. V. Hormonal interaction in the regulation of granulation tissue formation, *Endocrinology*, **51**:183, 1952.
284. TEILUM, G. Collagen diseases, *Lancet*, **2**:166, 1951.
285. TUNBRIDGE, R. E.; TATTERSALL, R. N.; HALL, D. A.; ASTBURY, W. T.; and REED, R. The fibrous structure of normal and abnormal human skin, *Clin. Sc.*, **11**:315, 1952.
286. UNNA, P. Basophiles Kollagen, Kollastin und Kollacin, *Monatsh. f. prakt. Dermat.*, **19**:465, 1894.
287. UPTON, A. C., and COON, W. W. Effects of cortisone and adrenocorticotrophic hormone on wound healing in normal and scorbutic guinea pigs, *Proc. Soc. Exper. Biol. & Med.*, **77**:153, 1951.
288. VANAMEE, P., and PORTER, K. R. Observations with the electron microscope on the solvation and reconstitution of collagen, *J. Exper. Med.*, **94**:255, 1951.
289. VAN LIER, E. H. B. Über die interfibrilläre Substanz der Lederhaut bei Säugetieren, *Ztschr. f. physiol. Chem.*, **61**:177, 1909.
290. VAUBEL, E. The form and function of syn-

- ovial cells in tissue cultures. I. Morphology of the cells under varying conditions. II. The production of mucin, *J. Exper. Med.*, **58**:63 and 85, 1933.
291. WATSON, E. M., and PEARCE, R. H. The mucopolysaccharide content of the skin in localized (pretibial) myxedema. II, *Am. J. Clin. Path.*, **19**:442, 1949.
 292. WAUGH, D. F. Physical properties of protoplasm, *Ann. Rev. Physiol.*, **14**:13, 1952.
 293. WELLS, G. C. Primary systematized amyloidosis with macroglossia, *Brit. J. Dermat.*, **64**:169, 1952.
 294. WENNER, H. A.; GIBSON, D. M.; and JAQUES, R. Specificities of hyaluronidases formed by several groups of streptococci, *Proc. Soc. Exper. Biol. & Med.*, **76**:585, 1951.
 295. WERNER, S. C.; HAMILTON, H.; and FRANTZ, V. K. Some effects of ACTH in chronic thyroiditis and myxedema, *Proc. Second Clin. ACTH Conf.*, **2**:521, 1951.
 296. WINTER, C. A., and FLATAKER, L. Influence of cortisone and related steroids upon spreading effect of hyaluronidase, *Federation Proc.*, **9**:137, 1950.
 297. ———. Effect of steroids upon resistance of skin to intracutaneous injection, *Proc. Soc. Exper. Biol. & Med.*, **79**:312, 1952.
 298. WISLOCKI, G. B.; BUNTING, H.; and DEMPSEY, E. W. Metachromasia in mam-
malian tissue and its relationship to mucopolysaccharides, *Am. J. Anat.*, **81**:1, 1947.
 299. WOLBACH, S. B. Controlled formation of collagen and reticulum: a study of the source of intercellular substance in recovery from experimental scorbutus, *Am. J. Path.*, **9**:689, 1933.
 300. WOLBACH, S. B., and MADDOCK, C. L. Cortisone and matrix formation in experimental scorbutus and repair therefrom, with contributions to the pathology of experimental scorbutus, *Arch. Path.*, **53**:54, 1952.
 301. WOLMAN, M. Staining of lipoids by the periodic-acid-Schiff reaction, *Proc. Soc. Exper. Biol. & Med.*, **75**:583, 1950.
 302. WOLPERS, C. Der Strukturwandel der Kollagenquerstreifung, *Klin. Wchnschr.*, **28**:317, 1950.
 303. WYCKOFF, R. W. G. The fine structure of connective tissues. *In*: Transactions of the Third Connective Tissue Conference. New York: Josiah Macy, Jr., Foundation, 1952.
 304. ZUCKERMAN, S. The effect of sex hormones, cortin, and vasopressin on water-retention in the reproductive organs of monkeys, *J. Endocrinol.*, **1**:147, 1939.
 305. ZWEIFACH, B. W., and CHAMBERS, R. The action of hyaluronidase extracts on the capillary wall, *Ann. New York Acad. Sc.*, **52**:1047, 1950.

In this chapter, the text often mentions only the senior author in reference to publications. For co-authors consult bibliography and author index.

CHAPTER 19

Carbohydrates

I. GLYCOGEN AND ITS METABOLISM	465
II. GLUCOSE CONTENT	469
III. METABOLISM OF GLUCOSE	472
A. Glycolysis	472
B. Decarboxylation and Dismutation of Pyruvic Acid	472
1. Decarboxylation	472
2. Dismutation	472
C. Oxidation of Pyruvic Acid, Tricarboxylic Cycle, and Accumulation of Citric Acid in Skin	473
D. Direct Oxidation of Carbohydrates	476
E. Lack of Glucose Synthesis	476
F. Peculiarities of Carbohydrate Metabolism in the Skin	477
IV. GLUCOSE METABOLISM UNDER PATHOLOGICAL CONDITIONS AND ITS POSSIBLE CLINICAL SIGNIFICANCE	477

I. GLYCOGEN AND ITS METABOLISM

HISTOCHEMICAL evidence indicates that there is considerable storage of glycogen in human and animal skin in the first half of fetal life (45). In a very remarkable manner the epidermis stores glycogen as long as the liver has not taken up this function. In the earliest stages all epidermal cells, excepting those in mitotic division, contain much glycogen (52). With the development of multiple layers in the epidermis, the amount of glycogen gradually diminishes. According to old data (45), the glycogen content of the skin in the newborn is from only 0.006 to 0.05 per cent of the wet weight.

Whereas in extrauterine life the mucous layer contains only small amounts of glycogen, it can be demonstrated histochemically with great ease in several differentiated elements of the normal skin throughout life. Many components of the hair follicles are rich in glycogen (34), and the external root

sheath cells, in particular, are "virtually packed" with it (34). The presence of glycogen in eccrine (and apocrine?) sweat glands and in sebaceous glands was mentioned on pages 190 and 331. Probably the most important sites of glycogen in the skin are the keratogenous zones of epidermis and hair. The possible significance of glycogen synthesis for the keratinization process was discussed in detail in chapter 16 (p. 373).

It has remained unexplained that although glycogen cannot be demonstrated histochemically in fully keratinized materials, hair contains large amounts of it. Bolliger and McDonald (8) found 400 mg. per cent of glycogen in the aqueous extracts of rabbit hair (p. 374). According to these authors, it could be the glycogen granules in the lower half of the outer root sheath of the hair which become deposited in or on the keratin of the hair fiber. Still, Bolliger (7) emphasizes that the glycogen found in the

hair fiber has apparently lost the staining properties it possesses in the root sheath and becomes demonstrable in the hair shaft only when isolated in hair extracts. Possibly, glycogen is bound chemically by keratin, so that it loses its histochemical color reactions, but it can be liberated by extraction with water. Thus the old problem of whether all the glycogen in the skin can be demonstrated histochemically or whether it also occurs in a "masked" form is still not settled.

According to most workers, all of whom used different methods of chemical determination, the total glycogen content of the skin in the adult organism is small. From the available data (13, 21), omitting early and obviously unreliable figures, one can calculate for man, dog, rabbit, and rat an average of 0.08 per cent (or 80 mg. per cent) of glycogen as related to wet weight of total skin. Cornbleet's range is from 68.1 to 84.7 mg. per cent (13).

It was reported that there is more glycogen in the "upper" than in the "lower" skin layers. Apparently, the epidermal glycogen, even in extrauterine life, amounts to more than the glycogen of the corium and that of the deeper parts of epithelial appendages put together. Fahrig (15) halved human skin into an upper layer containing epidermis and a lower layer containing fat. The upper layer had 0.16 per cent glycogen, the lower layer 0.07 per cent. Cornbleet (13) divided skin specimens from 20 persons into two equal parts horizontally. The superficial layer contained 0.075 per cent glycogen, and the deep layer 0.047 per cent, on the average. In neither of these experiments were the three main layers—epidermis, dermis, and subdermis—clearly separated from each other. Fahrig (15) speaks of the glycogen content of the "epidermis" and reports extremely high values in certain sites (0.23 per cent in epidermis of the skin of the breast, 0.34 per cent in epidermis of the skin of the leg), but without stating how he separated the "epidermis." In addition, the absolute amounts of glycogen in all these instances are extremely small, and the ac-

curacy of the methods of determination is open to question.

With regard to the distribution of the total glycogen in the body of the adult, the amount of glycogen in the skin is negligible. In a man weighing 70 kg. and with the skin representing about 16 per cent of this weight (p. 494), the 0.08 per cent glycogen represents 9 gm., as contrasted with average values of 245 gm. in the muscle and 108 gm. in the liver.

It has been a controversial issue whether the adult skin is able to build up glycogen and to break it down to glucose as muscle and liver do. Glucose must be phosphorylated in order to form glycogen; and this phosphorylation is catalyzed by the enzyme hexokinase. Since hexokinase is present in the skin (p. 475), the possibility that glycogen may be synthesized and hydrolyzed in the skin cannot be doubted. Such a mechanism is certainly operating in the arrector muscles and in eccrine glands (p. 190) and possibly also in the keratogenous zones (p. 373). But it is controversial whether glycogen is synthesized in the skin in consequence of chronic overfeeding or when, in acute experiments, excessive amounts of glucose are administered.

In contrast to early data from Pflüger's laboratory, indicating that gross overfeeding of dogs is followed by excessive glycogen storage in the skin (45), Pillsbury and Sternberg (42) found that in dogs on a high-carbohydrate diet the glycogen content of the skin was only a little higher than on a normal diet, the average values being 0.129 and 0.10 per cent, respectively, although the difference in the respective diets was great enough to cause a very substantial difference in the glycogen content of the livers. Nara-hara (35) found only slight variations in the cutaneous glycogen in rabbits after long-term feeding of glucose; in these experiments the glucose, but not the glycogen, content of the skin increased under the influence of the diet.

In acute experiments, after intravenous injections of glucose in dogs and in rabbits, none of the workers found increased glyco-

gen content in the skin (16, 29, 35, 13). A remarkable exception was one experiment of Matsumoto (29) in which 0.8 gm. of glucose was given intravenously to a rabbit. In this experiment, after the injection, the cutaneous glycogen rose from 83 to 133 mg. per 100 gm. of tissue in 1 hour and was back to 74 mg. in 2 hours. The cutaneous sugar in the same experiment rose from 123 to 251 mg. per 100 gm. of tissue.

In Cornbleet's experiments (13) measures which caused a substantial increase in the glucose content of the skin, such as intravenous injections of epinephrine and histamine, local heating, and locally applied inflammatory stimuli, failed to increase the amount of glycogen in the skin.

Conversely, measures decreasing the sugar content of the skin failed to decrease the amount of cutaneous glycogen. After injection of insulin into dogs, when the average glucose content of the skin fell from 67 to 40 mg. per cent, the glycogen did not show any decrease (13). The hepatic glycogen is known to decrease after insulin administration in normal animals (10, 12). As the cutaneous glycogen does not respond in this way, one may conclude that not only the synthesis but possibly also the degradation of glycogen are rather sluggishly performed functions of the skin.

Cornbleet (13) found no essential changes in cutaneous glycogen after elimination of the hepatic circulation or after phlorhizin injections, although the sugar concentration in the skin was decreased after these measures.

It was claimed that after removal of the skin there is a post mortem increase in the glucose content of the skin, indicating post mortem glycogenolysis (37, 61). However, Pillsbury (39) found in his *in vitro* experiments that very much less lactic acid is formed on the addition of glycogen to skin tissue than on the addition of glucose, an indication that glycogenolysis must be slight in removed pieces of skin, at least as far as added glycogen is concerned. Pillsbury and Sternberg (42) concluded that glycogenolysis after removal of the skin is a slow, non-

conspicuous process, as contrasted with the vigorous decomposition of glycogen in muscle after death.

Interestingly enough, it seems that glycogen can be forced to break down in the skin if diastatic (starch-splitting) enzymes are artificially introduced. Ottenstein (36) injected diastase into animals parenterally and found an increase in the amount of cutaneous glucose after such injections. This is difficult to understand, considering that the skin itself has considerable diastatic activity (45, 36, 47). It is not known whether the glycogen of the skin can be attacked by the skin's own diastase. Older data indicate that most of the diastatic activity is located in the epidermis, which is also the main site of glycogen (45). Still it is possible that substrate and enzyme are separated from each other in the same cell and that action occurs only in special cases of excitation. An effective method of breaking down glycogen in the skin seems to be excessive cooling. Gualdi and Baldino (19) found, on cooling, a decrease of cutaneous glycogen content from 0.07 to 0.054 per cent, with a simultaneous increase in lactic acid of from 0.028 to 0.272 per cent.

It is controversial whether breakdown of glycogen in general requires phosphorylation, as does its synthesis, or whether at least some phases of the breakdown are carried out by simple, diastase-type hydrolytic enzymes (11). If the latter is the case, there is a possibility that the skin's own diastase controls its glycogen level.

The bulk of available data suggests that the amount of glycogen in the skin does not depend, or depends only to a slight degree, on the amount of glucose made available from the blood stream to the skin. Apparently, it does not vary according to the demands of either the skin or the organism. This, of course, is in contrast to the behavior of glycogen in liver and muscle, tissues in which formation and decomposition of glycogen are essential parts of carbohydrate metabolism. In the skin, synthesis and decomposition of glycogen are apparently rather slow and weak processes, as if

they were rudimentary functions. What we know so far about glycogen in the skin probably fits best the old pathologic concept of "sessile" glycogen, as contrasted with "labile" glycogen. Long ago von Gierke (17) described sessile glycogen as a fixed constituent of the protoplasm, found mainly in tissues situated somewhat distant from the blood stream, such as cartilage and stratified epithelium. Also Lubarsch (26) mentioned that epithelial cells are preferred sites for deposition of glycogen. This sessile glycogen is not continuously synthesized and decomposed. It seems to be more firmly attached to other constituents of the protoplasm than the labile glycogen. Hanawa (22) claimed that it is more difficult to extract glycogen from tissue sections of the skin than from sections of the liver.

Smooth-muscle elements of the skin, secretory sweat-gland cells, and the cells of the keratogenous zone are, of course, excluded from these considerations. The function of glycogen in cutaneous smooth muscles is obviously the same as that of glycogen in other muscles. Concerning sweat glands, Gualdi and Baldino (19), Yuyama (68), and Shelley and Mescon (49) observed microscopically the disappearance of glycogen from secreting sweat-gland cells during activity, an indication that glycogen in sweat glands is functional (nonsessile), i.e., taking part in the physiological functions of the organ (p. 190).

In contrast to all previous data, Wohnlich (67) recently reported on an amazingly great ability of the skin of cattle and of man to synthesize glycogen from lactic acid in vitro. His extremely variable initial values of from 0.5 to 89.5 mg. per cent of glycogen in cattle skin and from 11.2 to 53.7 mg. per cent of glycogen in human skin rose, on the average, to two or three times the original value after a 2-hour incubation with lactate in the presence of oxygen. In single experiments, up to twenty-fold increases were

noted. Wohnlich calculates that the percentage increase of glycogen content in the skin, if incubated with lactate, is about ten times greater than that of the muscle under similar conditions. He considers the possibility that the skin, "like a sponge," takes up some of the lactic acid from sweat (p. 210) and "resynthesizes" it to glycogen. Should Wohnlich's findings be confirmed, our concept of the relative insignificance of glycogen in the carbohydrate metabolism of the skin will have to be reviewed.

Anyhow, it must be stated that the present state of our knowledge concerning the fate of glycogen in the skin is rather unsatisfactory. First, most workers have dealt with minute amounts of glycogen because of its low concentration in the skin, and this raises the question of the accuracy of the different methods used. The significance of differences or equalities under different experimental conditions has never been calculated in relation to the experimental error. Second, even if the relative constancy of the glycogen level, as presented in the foregoing paragraphs, is established beyond doubt, the possibility still remains that the constant glycogen level is not due to an inert metabolism but to a homeostatic regulating mechanism, whereby glycogen is continuously decomposed and synthesized but cannot be accumulated beyond a certain level, possibly by virtue of the regulating effect of diastatic enzymes. It is regrettable that this problem has not yet been attacked by the isotope technic. The administration of radioactive glucose or pyruvate, with subsequent analysis of cutaneous glycogen, may solve the problem.

Histochemical findings indicate that glycogen accumulates in the epidermis in pathological states connected with tissue damage and tissue repair. It accumulates also in inflammatory processes, particularly in states with enhanced (benign and malignant) epidermal proliferation (45, 1).

II. GLUCOSE CONTENT

Urbach and Fantl (58) were the first to apply the Hagedorn-Jensen method (20) for estimating glucose in the skin. Although this method, as applied to the skin, was criticized by Schaaf and Burckhardt (46), Pillsbury and Kulchar (40) in a detailed study found it to be reliable. Later on, Urbach and Lentz (59) found that, with their own method, part of the tissue sugar escaped detection; and from then on they applied the method of Rappaport (43), which yielded about 20 per cent higher values in man than the previous method had. It ap-

is a great amount of connective-tissue ground substance in the skin. Nevertheless, it is a remarkable finding of Urbach (60, 59) that the amount of "bound sugar" in the skin is considerably higher in diabetics and in pancreatectomized dogs than under normal conditions.

In human skin, taken as a whole without subcutaneous fat tissue, the fasting glucose values as related to wet weight are, according to all investigators, at a lower concentration than the percentage weight in the blood. The average concentration in the

TABLE 1
GLUCOSE CONTENT OF THE SKIN (13)

YEAR	AUTHORS	GLUCOSE (MG/100 GM WET WEIGHT)			
		Man	Dog	Rabbit	Mouse
1927.....	Folin <i>et al.</i> (16)		67		
1929.....	Urbach and Sicher (61)	47	60	117	53
1931.....	Trimble and Carey (54)	56			
1932.....	Urbach and Rejtö (60)	47	84	134	53
1933.....	Matsumoto (29)			112	
1933.....	Matsumoto (quoted in 13)		91	114	
1934.....	Narahara (35)			115	
1934.....	Pillsbury and Kulchar (41)			105	
1934.....	Moncorps <i>et al.</i> (33)				81.3
1937.....	Urbach <i>et al.</i> (57)	61	101	155	69
1940.....	Cornbleet (13)	60.2-81.5	71.3	96.9	

pears that the methods in this field require thorough re-evaluation.

The present section deals only with what has been called "free sugar," i.e., the amount of reducing substances obtained by Folin's (16) and Urbach's (58, 59) methods. The "bound sugar," meaning the amount of reducing substances obtained after acid hydrolysis of the skin, includes the carbohydrate components of mucoproteins in the connective-tissue ground substance (p. 422), an element which has hardly anything to do with the intrinsic metabolism of carbohydrates. In man's skin the amount of "bound sugar" was found to be fifteen times that of free sugar (60). This can be taken merely as confirmation of the fact that there

skin is two-thirds that in the blood. Capillaries are easily permeable to glucose, and the fall in concentration from blood to skin can be explained by the fact that glucose is utilized in the skin.

However, the concentration of glucose in the skin of common laboratory animals (dogs, cats, rabbits, guinea pigs, and rats) may be as high as, or higher than, that in the blood (59, 13). Possibly the skin is able to "store" sugar at sites where it is not immediately metabolized.

Numerical data of the older literature on fasting values in the skin as compared with the blood sugar are summarized by Rothman and Schaaf (45). Newer data have been reviewed by Cornbleet (13) (Table 1).

It has been claimed that although the relation of glucose concentration in blood and skin varies greatly according to species, it is rather constant within the same species (61).

It is a well-established fact that oral or parenteral administration of glucose increases the fasting glucose values of the skin in man and in animals (16, 61, 29, 48, 13). Indirectly, the same phenomenon was demonstrated by Pillsbury (39) when he found that the skin of well-fed rabbits yielded a greater amount of lactic acid than did the

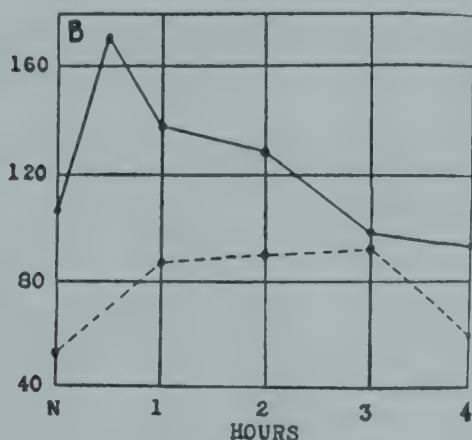


FIG. 1.—Normal sugar tolerance curve of the skin in man. The unbroken line indicates blood sugar, the broken line indicates skin sugar. Glucose expressed in mg. per cent. From Urbach and Lentz (59). (Reproduced by permission of the American Medical Association.)

skin of starving ones. The rise of the skin sugar level with hyperglycemia can be satisfactorily explained by simple diffusion through capillary vessels from blood into tissues. With hyperglycemia, the concentration difference between blood and tissue increases, and this results in a greater filtration rate from blood to tissue. However, in addition to a parallel increase in blood sugar and skin sugar, it was observed that in some instances the “concentration” of glucose in the skin exceeded that in the blood (16) and that after administration of glucose the cutaneous sugar stayed at a higher level for a considerably longer period of time than did the blood sugar, as compared with fasting values (61, 57, 59, 13). This discrepancy was quite clear-cut under physiological condi-

tions (Fig. 1) and was exaggerated in diabetes, in which fasting levels were found to be high (59).

Baisset *et al.* (2) advanced the thesis that after intravenous infusions glucose is not immediately taken up by the liver but is taken up by “peripheral tissues,” primarily by the skin. This peripheral storage, they said, is the reason for the short duration of hyperglycemia after intravenous injections of glucose. Later on, according to the authors, the peripherally stored glucose is again released into the blood stream and is then taken up by the liver. The authors attempted to prove this thesis by utilizing the discovery of Malméjac (27), who found that in the dog there is a skin area on the medial aspect of the thigh which is supplied by a purely cutaneous branch of the femoral artery; similarly, the corresponding vein collects blood only from the skin.

After intravenous injection of glucose these authors estimated the blood sugar simultaneously in blood from the carotid artery, from the cutaneous branch of the femoral vein, and from the femoral vein of the other side, where the cutaneous branch was ligated or the skin removed so that the femoral vein collected blood only from muscles. In about half the experiments, 15–30 minutes after injection a considerably lower blood sugar value was found in the cutaneous vein than in the femoral vein collecting blood from muscle only, an indication that the skin retained more sugar than did the muscle. Conversely, $1\frac{1}{2}$ hours after injection a “late hyperglycemia” was observed in the cutaneous-venous blood: 145 mg. per cent as contrasted with 90 mg. per cent in both the arterial and the muscular-venous blood. This transient hyperglycemia in the cutaneous vein was interpreted as a sign of the shift from skin to liver. Although the temporary storage in the skin could not be demonstrated consistently, the authors thought it a predominant phenomenon in intravenous glucose administration.

The finding of Urbach (61) and of Toshima (53) that the fasting value of cutane-

ous sugar is higher in animals on a diet rich in carbohydrates than in animals on a carbohydrate-poor diet, while the blood sugar is at the same level in both groups, was interpreted as a sign of a more permanent storing capacity of the skin. However, this finding could not be confirmed by Pillsbury and Sternberg (42), who did not find consistent differences in the amount of cutaneous sugar after different types of feeding.

The injection of large doses of insulin considerably decreases the glucose content of the skin (31, 48, 13), but it cannot be reduced below a certain minimum level (61, 54, 48). This finding led to the conclusion that the skin is able to "store" sugar, notwithstanding the effect of insulin (48). Still, Myiake and Narahara (31) found extremely low premortal values of cutaneous glucose in insulin shock.

Certainly, all investigators agree that after peroral or parenteral administration of glucose there is a "lag" in the return of the cutaneous sugar to fasting values. There has been some controversy as to whether this lag means a true, even if temporary, storage in the skin and whether this is in contradiction to the assumption that changes in cutaneous sugar levels are due to a "simple diffusion process" (48, 13).

Storage of glucose in the epidermis or other cellular elements is improbable because added glucose is immediately metabolized by skin slices under all circumstances. However, there is a possibility that glucose, if offered in excess, is adsorbed for shorter or longer periods on extracellular elements, such as connective-tissue fibers or connective-tissue ground substance, which do not possess the enzyme systems that metabolize glucose. The loosely bound water in connective tissue (p. 499) may also retain glucose. That such "binding" may occur is supported by the experiments of Urbach and Rejtö (60). These authors found that the amount of "bound" sugar is increased in diabetes (p. 469). Such binding would ex-

plain the "lag" in the return to normal of cutaneous sugar in hyperglycemia; it would also explain the higher fasting values in well-fed animals, the apparent ability of the skin to withstand to some degree the glycolytic action of insulin ("the skin tries to store sugar against insulin effect") (48), and the mobilization of cutaneous sugar by epinephrine (48). Unfortunately, there has been no investigation of whether connective-tissue elements are able, and to what degree, to bind glucose. No matter whether our explanation is correct, it is obviously futile to discuss the problem of "true storage" versus "simple diffusion process." The adsorption and release of sugar from extracellular elements will always depend on the concentration difference between blood and tissue. The expression "true storage" is better reserved for storage in the form of glycogen, a process which seems to play a minor role in the skin of adults.

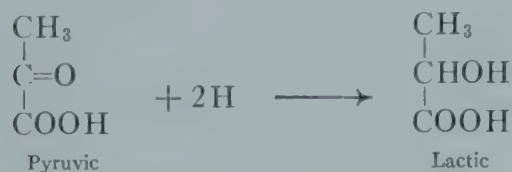
Obviously, it will be much easier to interpret the results of this kind of experimentation in the future when analyses are carried out in the epidermis and corium separately and when isotope technics are utilized.

The amount of glucose in the skin depends on its blood supply, i.e., on the rate of blood flow through the skin. In artificially produced inflammatory skin lesions, as well as in natural diseases with inflammatory processes, the glucose content is higher than in normal skin (33, 48, 13). However, if the damage is more severe, the skin seems to lose its sugar-retaining capacity (31).

Glucose was identified in the skin chemically as its phenylosazon compound by Schaaf and Burckhardt (46). The increase in the amount of reducing substances in the skin of diabetics was shown to be actually due to an increase in glucose (46, 55). In addition to glucose, Schaaf and Burckhardt (46) found some polyoses which had reducing sugar components other than glucose (glucosamine?).

III. METABOLISM OF GLUCOSE

The single steps in the breakdown of glucose to pyruvic acid are discussed in chapter 23 (p. 566). In anaerobic metabolism, "glycolysis" or "fermentation" takes place, in which lactic acid arises by the reduction of pyruvic acid:



In aerobic metabolism pyruvic acid is completely oxidized to carbon dioxide and water. In most tissues this oxidation comes about by the intervention of the tricarboxylic acid cycle of Krebs and its rather involved enzyme system (p. 567). In the skin, as in other organs, both the oxidative and the fermentative metabolism of glucose can be demonstrated.

A. GLYCOLYSIS

The formation of lactic acid from glucose by surviving skin was first demonstrated by Wohlgemuth and Klopstock (65), who found that this fermentation reaction in human skin is overwhelmingly a function of the epidermis. Slices of corium produced only negligible quantities of lactic acid. The data of Fahrigh (15) indicate that, on the addition of glucose and in the absence of oxygen, the epidermis has an even higher fermentation rate than has muscle.

Lactic acid is actually formed in the skin *in vivo*, and the rate of fermentation depends on the amount of glucose available. This was first demonstrated by Pillsbury (39), who found twice as much lactic acid in the skin of well-fed rabbits as in that of starved ones. He also showed that the skin of rabbits which had been given 3 gm. of glucose intravenously $\frac{1}{2}$ hour before the experiment produced twice as much lactic acid as did the controls.

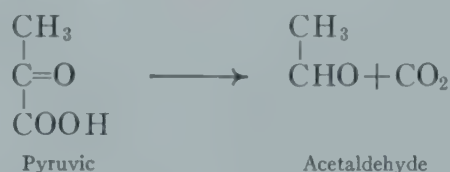
Comparing the oxidative and fermentative reactions of rat skin, Barron *et al.* (5) found that the oxidative reactions prevail

in fetal and juvenile skin, whereas in adult skin, in which oxidative reactions are less intense, aerobic and anaerobic processes are about equal: the "excess fermentation value" of Warburg is about zero in the skin of adult animals. Glycolysis by the skin of young rats is about five times greater than by the skin of adult animals. In human beings fetal skin forms about twice as much lactate as does adult skin (5).

B. DECARBOXYLATION AND DISMUTATION OF PYRUVIC ACID

1. Decarboxylation

One means of decomposition of pyruvic acid by tissues is its decarboxylation to acetaldehyde:



Previous data (66) indicated that the skin is able to perform this reaction. This would imply that the skin contains the carboxylase enzyme and its coenzyme, diphosphothiamine. But in a more recent work, Barron *et al.* (5) could not demonstrate the presence of acetaldehyde after a 3-hour incubation of 300 mg. of rat skin slices in Ringer-phosphate with pyruvate. Thus it seems improbable that this pathway is used by the cells of the skin.

In most tissues of thiamine-deficient animals the addition of thiamine increases the oxygen uptake and utilization of pyruvic acid (38, 4). This is not the case with the skin tissue of thiamine-deficient animals (5). The lack of action of thiamine in the skin was interpreted as an indication of a very slow rate of synthesis of the cocarboxylase diphosphothiamine from thiamine and phosphate (5).

2. Dismutation

"Dismutation" is a chemical reaction in which 2 molecules of the same compound

react to yield 1 oxidized and 1 reduced product. In the dismutation of pyruvic acid, 2 molecules of pyruvate yield 1 mole-

cule each of lactic acid, acetic acid, and carbon dioxide. This reaction does not require the presence of oxygen:



The skin has the necessary enzyme system to perform such dismutation. The degree of pyruvate dismutation under anaerobic conditions is about half the degree of pyruvate oxidation in the presence of oxygen. Both are about ten times greater in fetal rat skin than in adult rat skin, so that the oxidodismutation coefficient,

$$\frac{\text{Pyruvate utilized in O}_2}{\text{Pyruvate utilized in N}_2},$$

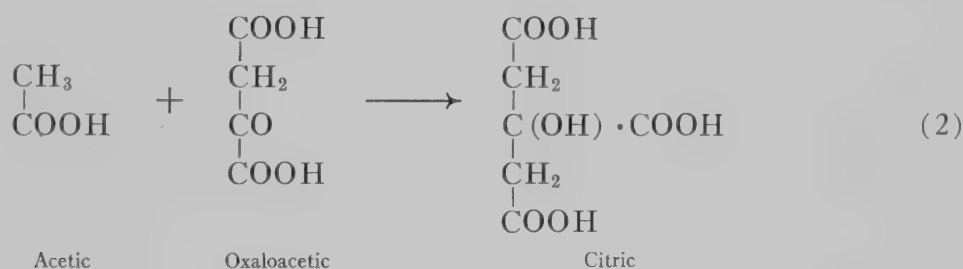
is similar in both fetal and adult skin (5).

C. OXIDATION OF PYRUVIC ACID, TRICARBOXYLIC CYCLE, AND ACCUMULATION OF CITRIC ACID IN SKIN

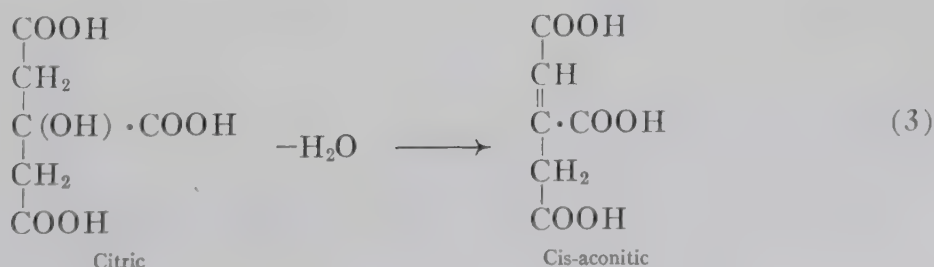
In aerobic metabolism the pyruvic acid formed by the degradation of glucose is oxidized to carbon dioxide and water. In liver and muscles this reaction takes place through the mediation of the tricarboxylic acid cycle of Krebs (3). The first step in this cycle is the oxidative decarboxylation of pyruvic acid to acetic acid:



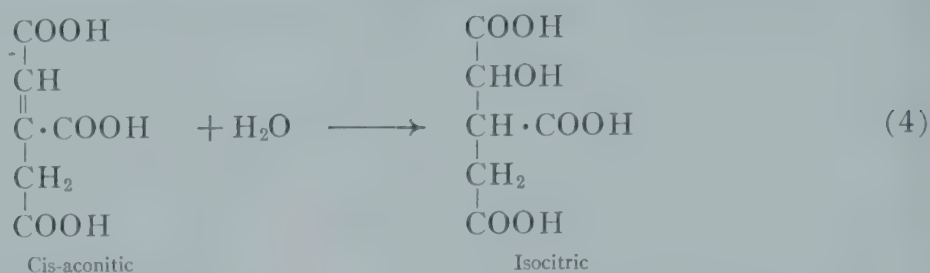
and the condensation of acetic acid with the dicarboxylic acid: oxaloacetic to the tricarboxylic acid: citric:



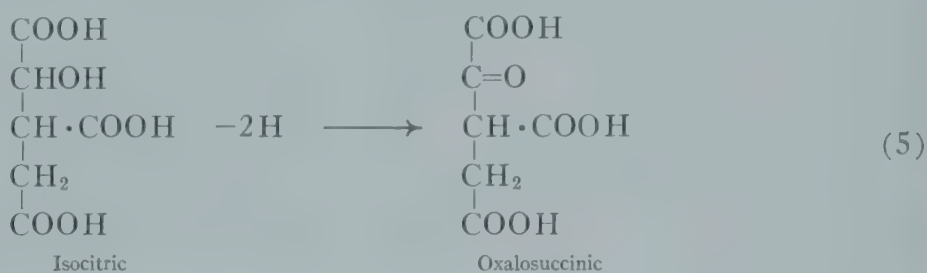
Citric acid transforms into the unsaturated tricarboxylic acid: cis-aconitic by loss of a water molecule:



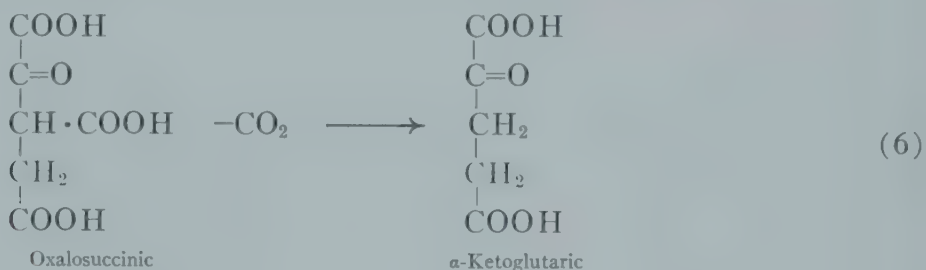
Cis-aconitic takes up water again, although not to form citric acid but an isomer of it, isocitric acid:



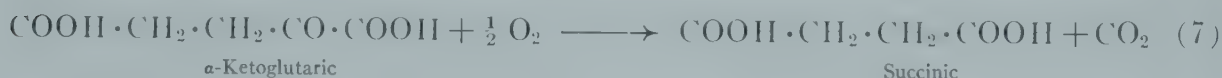
The reversible conversion of citric into isocitric via cis-aconitic is catalyzed by the enzyme aconitase. Isocitric is dehydrogenated to oxalosuccinic acid:



Oxalosuccinic is decarboxylated to the dicarboxylic α -ketoglutaric acid:



α -Ketoglutaric undergoes oxidative decarboxylation to succinic acid:



succinic is dehydrogenated to fumaric:



and fumaric takes up water to form malic acid:



Malic is dehydrogenated to oxaloacetic:



which may then react with a new molecule of acetic acid and repeat the cycle. All steps in this cycle are catalyzed by specific enzyme systems (p. 567).

The work of Barron *et al.* (5) indicates that this cycle does *not* operate in the skin. These authors found that the addition of citrate and of α -ketoglutarate to rat skin slices did not increase their oxygen uptake, as is the case with liver or muscle slices, which do utilize these compounds in the cycle.¹ Furthermore, they found that the enzyme isocitric dehydrogenase, which is required in the cycle to oxidize isocitric to oxalosuccinic acid (step 5), was not present in the skin. However, they found considerable oxygen uptake on the addition of succinate, and they concluded that possibly the carbohydrate metabolism of the skin utilizes only the short-cut reversible succinate-fumarate system (step 8) for the necessary oxidoreductive reactions. The succino-dehydrogenase enzyme is present in the skin (p. 571).

Barron's findings are particularly remarkable in view of the fact that citric acid is accumulated in the skin. Bones and skin are the only tissues which store citric acid. The citric acid accumulation in skin can now be explained by the finding that skin tissue does not utilize citric acid, while other tissues do. The source of citric acid in the skin could be the citric acid of the blood, which diffuses into the skin and is stored there because it is not being metabolized.

However, there is another possibility. Citric acid may accumulate in muscle if there is not enough oxygen available to carry on the Krebs cycle. If pyruvate and oxaloacetate are incubated with minced muscle under strict anaerobic conditions, substantial amounts of citric acid are formed (3) and accumulated (step 3).

Dickens (14) offers two sets of data which seem to support the hypothesis that citric acid is actually formed anaerobically in the skin. The first group of data indicates that

1. Most recently, R. D. Griesemer also reported that citrate is not metabolized by rat epidermis. (Annual Meeting, Soc. Invest. Dermat., May, 1953).

hair contains approximately ten times more citric acid than does total skin (Table 2). It might easily be that the particular type of cell death represented by the keratinization process (chap. 16) is associated with the gradual denaturation of oxidative enzymes and/or with lack of oxygen. Pyruvate and oxaloacetate may condense during keratinization to citric acid, as happens in muscle under anaerobic conditions.

Recently, confirming the relatively high citric acid content of keratinizing structures, Bolliger (7) reported the presence of

TABLE 2
CITRIC ACID CONTENT OF SKIN AND
FUR IN ADULT MICE (14)

	CITRIC ACID CONTENT (MG/100 GM OF WET WEIGHT)*	
	1st Sample	2d Sample
Skin with fur mostly removed	12.2	9.7
Fur alone	133.0	114.0

* The difference in the citric acid content of fur and skin, however, might be due partly to the difference in water content of total skin and hair, and data should be obtained in reference to dry weight before an actual increase of citric acid content during keratinization can be claimed.

50–60 mg. of citric acid in hot aqueous extracts of 100 gm. of rabbit hair (p. 374).

The second set of Dickens' data (14) indicates that in malignant proliferation of epithelium the citric acid accumulation is greater than in normal tissue (Table 3). This fact again points to the possibility that citric acid is formed anaerobically in the skin, because it is known that anaerobic metabolism is more prevalent in malignant growth than in normal epithelial tissue.

Miller and Carruthers (30) estimated the citric acid content of the separated epidermis of mice after shaving and found twelve to twenty-five times higher concentrations as related to wet weight than in other organs (Table 4). The same authors found that in all malignant tissues the citric acid concentration increased considerably, as

compared with the homologous parent-tissue, except in cutaneous squamous-cell carcinomas, the citric concentration of which is much lower than that of normal epidermis, which has an exceedingly high concentration to start with.

In malignant degeneration of the epidermis the decrease in citric acid concentration is paralleled by a decrease in calcium concentration (30). It was considered that

TABLE 3*
CITRIC ACID CONTENT OF NORMAL AND
MALIGNANT TISSUE OF VULVA IN
HUMAN FEMALE (14)

	CITRIC ACID CONTENT (MG/100 GM OF WET WEIGHT)	
	1st Sample	2d Sample
Carcinoma of vulva.....	8.8	7.0
Leukoplakia of vulva.....	8.2
Adjacent normal skin.....	5.3

* All specimens were taken from the same person.

TABLE 4
CITRIC ACID CONTENT OF
MOUSE ORGANS (30)

	Mg/Gm Wet Weight
Shaved epidermis.....	465
Liver.....	11.2
Muscle.....	30.0
Blood.....	29.1

possibly the two constituents are bound together as a diffusible complex and that calcium is carried away with this complex into the blood stream in carcinogenesis. However, it was also emphasized that in malignancies produced by local methylcholanthrene treatments the basal layer remains unchanged, while the rete and granular layer undergo hyperplasia, and that therefore there is a possibility that the chemistry of the cells actually does not change. It might be that the basal layer contains very much, the other layers only a little, of these substances; then the shift in analytical values is due to the changed proportions of the

different layers. This interpretation was called the "morphological hypothesis," as contrasted with the chemical one (30).

Citric acid can be stored only at sites where there is a lack of enzymes carrying the cycle from the citric acid stage to cis-aconitic, isocitric, etc. (steps 3, 4). Traces of aconitase activity were found in rat skin by Barron *et al.* (5); but these authors assume that the traces of the enzyme originated from minimal amounts of muscle tissue remaining on the skin samples. Thus the hypothesis of anaerobic citric acid synthesis in the skin is not in contradiction to the available data.²

D. DIRECT OXIDATION OF CARBOHYDRATES

From experiments with enzyme inhibitors, Barron *et al.* (5) concluded that carbohydrate metabolism in the skin may proceed not only via the glycolytic-oxidative pathway but also through direct oxidation. Iodoacetate and glyceraldehyde, which inhibit glycolytic enzymes, did not inhibit the respiration of rat skin slices, as they would if glucose utilization proceeded only by glycolysis to pyruvate and oxidatively from there on. The pathway of such direct oxidation is unknown. However, oxygen uptake in the presence of carbohydrate enzyme inhibitors could be due to fatty-acid oxidation, and thus it is not certain that "direct oxidation" of carbohydrates does take place in the skin.

E. LACK OF GLUCOSE SYNTHESIS

All steps in the glycolytic-oxidative degradation of glucose are reversible, because the enzymes catalyzing these steps of degradation also catalyze the reactions in the opposite direction. Thus synthesis of glucose from pyruvate by kidney or liver slices can be easily demonstrated. When, however,

2. Concerning nonutilization of citric acid by the skin, Dr. A. B. Lerner, in his chapter on "Enzymes" (p. 568), gives a different interpretation of the available data. Instead of trying to "adjust" the different viewpoints, I prefer to let both interpretations stand as they are.

skin slices from rat fetuses and adult rats were incubated with pyruvate, no such carbohydrate synthesis could be demonstrated (5). These findings are in contradiction to those of Wohnlich (67).

F. PECULIARITIES OF CARBOHYDRATE METABOLISM IN THE SKIN

The work of Barron *et al.* (5) indicates that the carbohydrate metabolism of the skin may be distinguished by three peculiar features: (1) the tricarboxylic acid cycle is not operating; (2) carbohydrate synthesis from pyruvate does not take place; and (3) formation of cocarboxylase from thiamine and phosphoric acid is slow or absent. Possibly these three features can be regarded as deficiencies in the elaborate enzyme systems of carbohydrate metabolism. It has been assumed that this deficiency might be the reason for the skin's being particularly vulnerable to inadequate nutrition. Indeed, in many nutritional deficiencies, particularly in deficiencies of B factors (required for carbohydrate metabolism), the skin is the first organ to display clinical disease (45). A further peculiarity is that although the skin

contains glycogen, it is not at all, or only slightly, involved in metabolism and thus possibly represents a so-called "sessile" type of glycogen.

Finally, it appears that the skin is very active in forming lactic acid from glucose. According to Fahrig (15), anaerobic lactate formation is greater in the epidermis than in muscle tissue. In the presence of oxygen, less lactic acid is formed than under anaerobic conditions. This is true for the skin (65, 15, 5), as well as for all normal tissues. Nevertheless, the data of Fahrig (15) and of Pillsbury (39) suggest that the amounts formed, even in the presence of oxygen, are fairly high. However, the skin does not approach the peculiar metabolism of malignant tumors in which aerobic and anaerobic lactate formation is approximately equal (62, 63). The finding of Pillsbury (39) that there is no difference in the amount of lactate formed under aerobic and anaerobic conditions has not been duplicated. Similarly, the interesting claim of Wohlgemuth and Ikebata (64), that glycolysis in the skin is greatly reduced by ultraviolet and X-ray irradiations, requires confirmation.

IV. GLUCOSE METABOLISM UNDER PATHOLOGICAL CONDITIONS AND ITS POSSIBLE CLINICAL SIGNIFICANCE

In human diabetes and in pancreatectomized dogs the sugar content of the skin is high. The "skin glucose tolerance test" as practiced by Urbach³ (56, 59) reveals a "diabetic type of curve," with an unusually steep and high rise of the skin sugar, delayed maximum, and delayed return to fasting values. This delay is more pronounced in the skin than in the blood. The peak glucose level is reached in the blood after 2 hours and in the skin after 3 hours or more; the skin sugar remains at a high level at least 1 hour longer than does the blood sugar. In pancreatectomized dogs this sluggishness of the skin sugar curve is even more pronounced than in man.

Urbach placed emphasis on the occurrence of cases in which blood sugar values were normal but fasting skin sugar levels

abnormally high. This discrepancy was even greater when the glucose tolerance tests were compared, and a pathological skin glucose curve was found, along with a normal blood sugar curve. For this situation Urbach coined the terms "cutaneous glycohistechia" and "skin diabetes" (56, 59). He thought that this situation played an important pathogenetic role not only in bacterial and fungus infections, which are known to have a high incidence in diabetes, but also in some "eczemas" in intertriginous localization and in some cases of pruritus. Some clinical evidence was offered to support this thesis.

3. After removing a fasting biopsy specimen from the skin, 100 gm. glucose are given perorally, and biopsy specimens are taken again 1, 3, and 4 hours after the ingestion of glucose.

In contrast to the frankly "diabetic" glucose tolerance curves, Urbach differentiated an abnormal blood glucose tolerance curve, with an unusually great and steep rise but a rapid return, accompanied by a normal skin glucose tolerance curve. He called these abnormal blood sugar curves those of the "sympathetic-endocrine type" and stressed the point that they do not indicate diabetes or latent diabetes and cannot be made responsible for any cutaneous manifestations. Modern students of diabetes, of course, are inclined to believe that a steep and high rise of the tolerance curve with normal rapid return is without any clinical significance. Urbach's work has never been duplicated, and its significance, physiologically and clinically, remains to be established.

The only clear-cut clinical fact which can be related to the changed chemical composition of the skin in diabetes is the greatly increased susceptibility to staphylococcal and monilial infections and the greater severity of these infections in diabetics. This clinical observation was substantiated experimentally by Pillsbury and Sternberg (42), who found that experimental pyogenic infections were definitely more severe in dogs on a high-carbohydrate intake than in those on either a low-carbohydrate or a high-fat diet or in fasting dogs. The interpretation of this phenomenon is still controversial. The simplest explanation, of course, would be that skin with a higher glucose content offers a better culture medium for these organisms than does normal skin. However, it was pointed out by Pillsbury and Kulchar (41), with reference to several data in the older literature, that some patients with severe diabetes may have an entirely normal resistance to infection and that thus the increased amount of glucose in the skin is apparently not the sole reason for the decreased resistance to infection. Pillsbury (39) mentioned older views, according to which an intermediate in glucose metabolism might be incriminated for diabetic skin manifestations, and he speculated that possibly lactic acid could be this intermediate,

particularly because data are available to show that lactic acid promotes bacterial growth, is toxic for leukocytes, and promotes inflammation as well. Later on, however, the interpretation of the influence of excess carbohydrate on infections has been that it is exerted through its effect on the water content of the skin (23, 42). Increased carbohydrate intake leads to water retention (41), while carbohydrate restriction results in dehydration. Kulchar and Alderson (23) showed that dehydration effectively decreases susceptibility to experimental infections of the skin.

In the discussion of the problem of increased susceptibility to infections in diabetes and in excess carbohydrate feeding, the chemical composition of the skin surface film, as contrasted with the chemical composition of the skin tissue, has not been considered. The skin surface film extends into the horny layer and into the follicular canal. An increase in the amount of glucose in this film (as related to surface) might—at least theoretically—promote the take and thriving of *Candida albicans* in the horny layer and of staphylococci in the follicular canal. Particularly, if it were shown unequivocally that sweat in diabetes and in excess carbohydrate feeding contains more glucose than normal, the direct promoting effect of increased amounts of glucose on exogenous infections still would remain a possibility.

Glucose excreted with the sweat (p. 209) remains on the skin surface after the water has evaporated. Up to recent times it seemed to be well established that the glucose content of sweat depends primarily on the level of the blood sugar (p. 209) and that therefore it is increased in diabetes and in excessive carbohydrate intake (28, 50). Talbert *et al.* (50) showed that the blood sugar can be lowered by profuse sweating, particularly if it is driven high by the administration of carbohydrates. Carrié and Koenig (9) specifically tested the skin film in diabetics and found abnormally large amounts of glucose. Thus it appears possible that the hitherto unexplained variations in susceptibility to infections in diabetics depends

primarily on the individually highly variable efficiency of sweat glands and on the amount of sweat actually secreted, according to atmospheric temperature, exercise, etc. However, lately Lobitz *et al.* (25, 24), collecting sweat from the palmar surface of fingertips, found no increased amounts of sugar in the sweat of diabetics (p. 209). Thus even the simple problem as to whether sugar accumulates on the skin surface in abnormal quantities when the supply from the blood is increased has not been definitely settled. This is just one more example of how much more simple experimentation remains to be done in the domain of investigative dermatology.

Not only glucose but lactic acid also may accumulate on the skin surface, again deriving from sweat. Lactic acid concentration in sweat is high but, at the same time, very variable (p. 211). The variations in the lactic acid content of the surface may play a role in variable susceptibility to infections.

If one attempts to evaluate the possible clinical significance in changes of water content in the skin under the influence of carbohydrate accumulation, one encounters the difficulty that water accumulates in the corium (p. 496) but not in epithelial tissue

and not on the surface. Thus if cutaneous infections by bacteria and fungi are facilitated and aggravated by increased water content, this might come into operation only after the organisms penetrated through the epidermis or appendages. They can take advantage of a high water content only in the corium.

Gross suggested that cutaneous manifestations of diabetes may be connected with a relative insufficiency in vitamins, because these manifestations sometimes can be controlled by vitamin and liver-extract administration (18).

In a remarkable series of experiments Monacelli *et al.* (32, 44) found that the glucose content of the uninvolved skin in psoriatic patients is considerably higher than normal. In the skin of normal persons the values were between 44 and 56 mg. per cent, with an average of 52 mg. per cent, while in the uninvolved skin in psoriasis the range was 65–105 mg. per cent, and the average was 79 mg. per cent glucose per wet weight of skin tissue. Monacelli and Ribuffo (32) also presented indirect evidence that the phosphorylation of glucose in the skin is deficient in psoriasis.

BIBLIOGRAPHY

1. ARGYRIS, T. S. Glycogen in the epidermis of mice painted with methylcholanthrene, *J. Nat. Cancer Inst.*, **12**:1159–65, 1952.
2. BAISET, E.; BUGUARD, L.; ROUZAND, J. J.; and LOULA, L. C. Le stockage lacunaire du glucose dans la peau, *J. de physiol. et de path. gén.*, **37**:1583–91, 1939–40.
3. BALDWIN, E. Dynamic aspects of biochemistry. Cambridge: At the University Press, 1949.
4. BARRON, E. S. G.; LYMAN, C. M.; LIPTON, M. A.; and GOLDINGER, J. M. Studies on biological oxidations. XVII. The effect of thiamine on the metabolism of alpha ketoglutarate, *J. Biol. Chem.*, **141**:975–79, 1941.
5. BARRON, E. S. G.; MEYER, J.; and BAKER MILLER, Z. The metabolism of the skin. Effect of vesicant agents, *J. Invest. Dermat.*, **11**:97–118, 1948.
6. BODO, R. C., and NEUWIRTH, I. The relation of insulin to liver glycogen, *J. Physiol.*, **103**:5–17, 1933.
7. BOLLIGER, A. Water extractable constituents of hair, *J. Invest. Dermat.*, **17**:79–84, 1951.
8. BOLLIGER, A., and McDONALD, N. D. The glycogen content of rabbit hair, *Australian J. Exper. Biol. & M. Sc.*, **26**:459–63, 1949.
9. CARRIÉ, C., and KOENIG, R. Über den Zuckergehalt auf der Haut bei Normalen und Diabetikern, *Arch. f. Dermat. u. Syph.*, **173**:611–14, 1936.
10. CORI, C. F. Insulin and liver glycogen, *J. Pharmacol. & Exper. Therap.*, **25**:1–33, 1925.
11. CORI, G. T., and LARNER, J. Action of amylo-1,6-glucosidase and phosphorylase on glycogen and amylopectin, *J. Biol. Chem.*, **188**:17–29, 1951.

12. CORKILL, B. The influence of insulin on the distribution of glycogen in normal animals, *Biochem. J.*, **24**:779-94, 1930.
13. CORNBLEET, T. Cutaneous carbohydrates. I. The normal skin, *Arch. Dermat. & Syph.*, **41**:193-213, 1940.
14. DICKENS, F. The citric acid content of animal tissues, with reference to its occurrence in bone and tumour, *Biochem. J.*, **35**:1011-23, 1941.
15. FAHRIG, C. Über den Kohlehydratumsatz der Geschwülste und ihrer Vergleichsgewebe sowie seine Beziehungen zum Milchsäuregehalt des Körpers, *Ztschr. f. Krebsforsch.*, **25**:146-228, 1927.
16. FOLIN, O.; TRIMBLE, H. C.; and NEWMAN, L. H. The distribution and recovery of glucose injected into animals, *J. Biol. Chem.*, **75**:263-81, 1927.
17. GIERKE, E. VON. Physiologische und pathologische Glykogenablagerung, *Ergebn. d. Path.*, **11**:871, 1907. Quoted by ROTHMAN and SCHAAF (45).
18. GROSS, P. The significance of nutritional deficiencies in the practice of dermatology, *Clinics*, **3**:789-812, 1944.
19. GUALDI, A., and BALDINO, N. Ricerche sul metabolismo per variazioni della temperatura locale dei tessuti; il contenuto in glicogeno e in acido lattico della cute e dei muscoli raffreddati, *Riv. di pat. sper.*, **5**:318-22, 1930.
20. HAGEDORN, H. C., and JENSEN, N. B. Zur Mikrobestimmung des Blutzuckers mittels Ferricyanid, *Biochem. Ztschr.*, **135**:46-58; **137**:92-95, 1923.
21. HALDI, J.; GIDDINGS, G.; and WYNN, W. Dietary control of the water content of the skin of the albino rat, *Am. J. Physiol.*, **135**:392-97, 1941-42.
22. HANAWA, S. Zur Kenntnis des Glykogens und des Eleidins in der Oberhaut, *Arch. f. Dermat. u. Syph.*, **118**:357, 1913. Quoted by ROTHMAN and SCHAAF (45).
23. KULCHAR, G. V., and ALDERSON, H. E. Relation of water metabolism to experimental skin infections, *Brit. J. Dermat.*, **48**:477-83, 1936.
24. LOBITZ, W. C., JR., and MASON, H. L. Discussion of studies on chloride, urea, glucose, uric acid, ammonia nitrogen, and creatine, *Arch. Dermat. & Syph.*, **57**:907-15, 1948.
25. LOBITZ, W. C., JR., and OSTERBERG, A. E. Chemistry of palmar sweat. III. Reducing substances (glucose), *Arch. Dermat. & Syph.*, **56**:819-26, 1947.
26. LUBARSCH, O. Über die Bedeutung der pathologischen Glykogenablagerungen, *Virchows Arch. f. path. Anat.*, **183**:188, 1906. Quoted by ROTHMAN and SCHAAF (45).
27. MALMÉJAC, J., and DESANTI, E. Technique permettant l'enregistrement des réactions vaso-motrices de la peau, *Compt. rend. Soc. de biol.*, **127**:542-46, 1938.
28. MARCHIONINI, A., and OTTENSTEIN, B. Stoffwechselveränderungen im Schwitzbad bei Hautgesunden und Hautkranken. II. Einfluss auf den Kohlehydrat und Lipidstoffwechsel, *Klin. Wchnschr.*, **10**:971-73, 1931.
29. MATSUMOTO, Y. An experimental study of the carbohydrates of the skin, with special reference to the combined sugar (protein sugar) and the glycogen. II, *Mitt. a. d. med. Akad. zu Kioto*, **8**:481, 1933.
30. MILLER, H. and CARRUTHERS, C. Citric acid metabolism in carcinogenesis and its relationship to calcium metabolism, *Cancer Research*, **10**:636-41, 1950.
31. MIYAKE, I., and NARAHARA, K. Eczematöse Hautveränderung und Zuckerstoffwechsel; Hautzucker und eczematöse Hautveränderung, *Jap. J. Dermat. & Urol. (abstr. sec.)*, **30**:85, 1930.
32. MONACELLI, M., and RIBUFFO, A. Der Hautzucker Gehalt bei der Psoriasis, *Hautarzt*, **3**:498-503, 1952.
33. MONCORPS, C.; BOHNSTEDT, R. M.; and SCHMID, R. Über den Zucker- und Glutathiongehalt von Blut und Haut bei Höhen- sonnen- und Crotonöldermitis; ein Beitrag zur Frage peripherisch bedingter Stoffwechselvorgänge, *Arch. f. Dermat. u. Syph.*, **169**:67-76, 1933/34.
34. MONTAGNA, W.; CHASE, H. B., and HAMILTON, J. B. The distribution of glycogen and lipids in human skin, *J. Invest. Dermat.*, **17**:147-57, 1951.
35. NARAHARA, K. Experimentelle Untersuchungen über den Hautzucker. IX. Hautglykogen und Zuckerabbau in der Haut, *Jap. J. Dermat. & Urol. (abstr. sec.)*, **35**:31, 1934.
36. OTTENSTEIN, B. Untersuchungen über den Gehalt der Haut und des Blutes an diastatischem Ferment und dessen biochemische Bedeutung bei Hautkrankheiten. I. Über das diastatische Ferment der Haut

- bei Hautgesunden, *Biochem. Ztschr.*, **240**: 328-43, 1931.
37. PALMER, W. W. The concentration of dextrose in the tissues of normal and diabetic animals, *J. Biol. Chem.*, **30**:79, 1917. Quoted by ROTHMAN and SCHAAF (45).
38. PETERS, R. A. Die Physiologie des Vitamin B₁, *Deutsche med. Wchnschr.*, **63**:1144-47, 1937.
39. PILLSBURY, D. M. The intrinsic carbohydrate metabolism of the skin. A preliminary report of experimental studies, *J.A.M.A.*, **96**:426-32, 1931.
40. PILLSBURY, D. M., and KULCHAR, G. V. The use of the Hagedorn-Jensen method in the determination of skin glucose, *J. Biol. Chem.*, **106**:351-56, 1934.
41. ———. The relation of experimental skin infection to carbohydrate metabolism. The effect of hypertonic glucose and sodium chloride solutions injected intraperitoneally, *Am. J. M. Sc.*, **190**:169-78, 1935.
42. PILLSBURY, D. M., and STERNBERG, T. H. Relation of diet to cutaneous infection, *Arch. Dermat. & Syph.*, **35**:893-909, 1937.
43. RAPPAPORT, F. *Mikrochemie des Blutes*. Vienna: E. Haim, 1936.
44. RIBUFFO, A. Metabolismo nella psoriasi. In: MONACELLI, "Psoriasi," pp. 51-111. Turin: Minerva Medica, 1952.
45. ROTHMAN, S., and SCHAAF, F. Die Chemie der Haut. In: JADASSOHN, *Handb. d. Haut- u. Geschlechtskr.*, **1/2**:161-377. Berlin: J. Springer, 1929.
46. SCHAAF, F., and BURCKHARDT, W. Versuche zum histologisch-chemischen Nachweis intravenös injizierten Substanzen (speziell 1-3,4-Dioxyphenylalanin) innerhalb der Epithelzellen. Anhang: Histologisch-chemischer Nachweis reduzierender wahrscheinlich zuckerartiger Substanzen in der Haut von Menschen und Tieren mit Silbernitrat, *Arch. f. Dermat. u. Syph.*, **165**:157-73, 1932.
47. SCHUPPLI, R. Über den Fermentstoffwechsel der Haut. I, *Dermatologica*, **93**:97-112, 1946.
48. SELLEI, C., and SPIERA, M. Die Rolle der Haut im Kohlehydratstoffwechsel, *Biochem. Ztschr.*, **296**:83-89, 1938.
49. SHELLEY, W. B., and MESCON, H. Histochemical demonstration of secretory activity in human eccrine sweat glands, *J. Invest. Dermat.*, **18**:289-301, 1952.
50. SILVER, S.; FORSTER, W.; and TALBERT, G. A. Simultaneous study of the constituents of the sweat, urine and blood, also gastric acidity and other manifestations resulting from sweating. VI. Sugar, *Am. J. Physiol.*, **84**:577-82, 1928.
51. STERNBERG, T. H., and PILLSBURY, D. M. The influence of avitaminosis A on experimentally produced cutaneous infections in rats, *Arch. Dermat. & Syph.*, **35**:247-50, 1937.
52. SUNDBERG, C. Das Glykogen in menschlichen Embryonen von 15, 27, und 40 mm., *Ztschr. f. Anat.*, **73**:168-246, 1924.
53. TOSHIMA, E. Beiträge zur experimentellen Untersuchung über den Hautzucker. II. Hautzucker bei der Zuckerstoffüberfütterung, *Jap. J. Dermat. & Urol. (abstr. sec.)*, **36**:67, 1934.
54. TRIMBLE, H., and CAREY, B. W., JR. On the true sugar content of skin and of muscle in diabetic and non-diabetic persons, *J. Biol. Chem.*, **90**:655-63, 1931.
55. ———. True sugar content of skin in diabetes, *Arch. Dermat. & Syph.*, **25**:6-10, 1932.
56. URBACH, E. Skin diabetes: hyperglycemia without hyperglycemia, *J.A.M.A.*, **129**:438-40, 1945.
57. URBACH, E.; DEPISCH, F.; and SICHER, G. Zum Problem des isolierten hohen Hautzuckers bzw. Hautdiabetes, *Klin. Wchnschr.*, **16**:452-56, 1937.
58. URBACH, E., and FANTL, P. Methoden zur quantitativ-chemischen Analyse der Haut. II. Der Zuckergehalt der normalen Haut, *Biochem. Ztschr.*, **196**:474-77, 1928.
59. URBACH, E., and LENTZ, J. W. Carbohydrate metabolism and the skin, *Arch. Dermat. & Syph.*, **52**:301-16, 1945.
60. URBACH, E., and REJTÖ, K. Beiträge zu einer physiologischen und pathologischen Chemie der Haut. XI. Über den freien und "gebundenen" Zucker in der Haut unter physiologischen, experimentell veränderten und pathologischen Bedingungen bei Mensch und Tier, *Arch. f. Dermat. u. Syph.*, **166**:478-90, 1932.
61. URBACH, E., and SICHER, G. Beiträge zu einer physiologischen und pathologischen Chemie der Haut. III. Der Zuckergehalt der Haut unter physiologischen und pathologischen Bedingungen, *Arch. f. Dermat. u. Syph.*, **157**:160-82, 1929.
62. WARBURG, O. Verbesserte Methode zur

- Messung der Atmung und Glykolyse, *Biochem. Ztschr.*, **152**:51-63, 1924.
63. WARBURG, O.; POSENER, K.; and NEGELEIN, E. Über den Stoffwechsel der Carcinomzelle, *Biochem. Ztschr.*, **152**:309-44, 1924.
64. WOHLGEMUTH, J., and IKEBATA, T. Die Fermente der Haut. VIII. Über die Milchsäurebildung in der Haut und ihre Beeinflussung durch verschiedene Strahlenarten, *Biochem. Ztschr.*, **186**:43-53, 1927.
65. WOHLGEMUTH, J., and KLOPSTOCK, E. Die Fermente der Haut. V. Atmung und Glykolyse der Haut und ihre Beeinflussung durch Hormone, *Biochem. Ztschr.*, **175**:202-15, 1926.
66. WOHLGEMUTH, J., and NAKAMURA, Y. Die Fermente der Haut. VI. Über das Verhalten der Lipase und über das Vorkommen von Phosphatase, Sulfatase und Carboxylase in der Haut, *Biochem. Ztschr.*, **175**:216-32, 1926.
67. WOHNLIICH, H. Zur Kohlehydratsynthese der Haut, *Arch. f. Dermat. u. Syph.*, **187**:53-60, 1948-49, and **188**:1-19, 1949-50.
68. YUYAMA, H. Über die histologische Untersuchung der Glykogenverteilung in der leprösen Haut, mit besonderer Berücksichtigung der Beziehung zwischen der Funktion der Schweissdrüsen und der Schwankung des Glykogens, *Jap. J. Dermat. & Urol.* (abstr. sec.), **37**:134-36, 1935.

CHAPTER 20

Lipid Constituents

I. TOTAL FAT CONTENT OF THE SKIN	483
II. TOTAL LIPID CONTENT OF THE EPIDERMIS	484
III. STEROLS	485
IV. PHOSPHOLIPIDS	488
V. CHOLINE	489

IT SHOULD be emphasized, possibly more so for the skin than for any other living tissue, that fatty substances in tissues consist of two different kinds of elements. One is the fat deposited between cells and used as fuel reserve. This portion consists mainly of glycerides of palmitic, stearic, and oleic acids, and it varies greatly with the nutritional state of the organism. The other part,

mainly sterols and phospholipids, are constant elements, independent of the nutritional state. They are part of the actual texture of cells and tissues and are present in a rather constant quantity and proportion in living tissues. They constitute a small percentage of the total fat. Therefore, data on total fat mirror primarily the amount of depot fat.

I. TOTAL FAT CONTENT OF THE SKIN

The total fat content of human skin (epidermis + corium) varies in a wide range, and it is hardly possible to establish "normal" average values. Urbach (50) found an average of 6 per cent of the wet weight of fresh skin to be fat, but he also stated that anything between 0.7 and 10 per cent can be normal. Nadel (25), too, found physiological variations between 0.3 and 10 per cent but claimed that the percentage is rarely more than 3.5. The fat content of the skin is quite obviously influenced by the nutritional state. If the subcutaneous tissue is poorly developed, there is less fat also in the skin proper (25). A remarkable feature was discovered by Kooyman (18) in the albino rat: the fat content of the skin varied inversely with the size of the animal; with striking regularity, the skin of large rats had the

lowest, that of small rats the highest, total fat content. There is less fat in the papillary layer + epidermis than in the reticular layer in human skin (50, 25). In the albino rat, the skin of females has a much higher fat content than that of males (15). This sex difference is highly significant also if the fat content is related to dry weight and can be demonstrated in various kinds of dietary regimes (15, 56). Whether this sex difference exists in humans is not known.

Just as the skin functions as a depot for water (p. 496), so it seems to be a reservoir for fat. Haldi *et al.* (15, 56), investigating the effect of different feeding forms in the rat, found large fat deposits in the skin of animals fed on a high-carbohydrate diet and an even higher fat content in the skin of animals fed on a high-fat diet. Concurrently, as

the water content and the protein content of the total skin diminished, the glycogen content remained essentially unchanged (Table 1). Conversely, as shown by Matthews, Newton, and Bloor (21), when fat is mobilized to the liver in pancreatectomized animals, the total fat loss, percentage-wise, is greatest from the skin.

However, this depot function for fats is not so clear as that for water. First, there is always an inverse correlation between fat and water content, and sometimes it is hard to figure out whether the change in water or

56) show a great and preferential increase in fat content of the skin proper on high-carbohydrate and high-fat intake, even if the values are calculated for dry substance. In Haldi's experiments adherent fat was removed from the skin samples.

In animals on a fat-free diet the skin (or skin plus subcutaneous tissue) seems to retain more fat than other organs do (18, 40). Smedley-MacLean and Hume (40) found an amazingly great ability of the skin to retain fat in rats which were kept on a fat-free diet. Even more surprising was the observation

TABLE 1*
WATER, FAT, PROTEIN, AND GLYCOGEN CONTENT OF SKIN
OF ALBINO RATS ON VARIOUS DIETS (15)

DIET FORM	MALES				FEMALES			
	Water	Fat†	Protein (N×6.25)	Glycogen	Water	Fat†	Protein (N×6.25)	Glycogen
Stock diet.....	60.3	6.6	29.6	0.073	51.1	12.0	25.0	0.064
High carbohydrate.	55.1	9.3	28.3	.069	43.7	20.1	23.5	.045
High fat.....	51.4	17.6	24.5	0.075	39.2	31.6	19.4	0.050

* Each value represents an average of 8 experiments. † As fatty acid.

in fat content is the primary event. Second, some of the pertinent experiments were done on skin specimens which were not clearly separated from the subcutaneous tissue. "Normal" values as high as 25-26 per cent fat in "skin" (21, 1) clearly indicate that subcutaneous fat must have been contained in "skin" specimens. Nevertheless, at least the dietary experiments of Haldi *et al.* (15,

that the longer the rats were fat-starved, the higher was the lipid content of their skin. The skin also contained most of the essential fatty acids (p. 318) in the animals which were kept on diets deficient in these acids. One could speculate that such essential fatty acids might be synthesized by sebaceous glands (p. 318).¹

II. TOTAL LIPID CONTENT OF THE EPIDERMIS

Total lipid content of mouse epidermis in relation to total nitrogen content was estimated by Wicks and Suntzeff (53). Values in six pooled samples ranged from 4.74 to 6.71 mg. and averaged 5.25 mg. total lipids per milligram of nitrogen. These are surprisingly high values, which mean that there is almost as much lipid in the epidermis as there is protein. If all nitrogen is taken as protein-nitrogen, the lipid/protein relation in the epidermis appears to be 1:1.2, while the

same relation for total rat skin, calculated from the data of Haldi *et al.* (15), is 1:4.5 in the male and 1:2.1 in the female.

1. Smedley-MacLean's deficiently fed rats kept on living but did not grow. Apparently, the tiny amounts of essential fatty acids retained were sufficient to allow maintenance of essential life-processes but did not permit new cell formation. The skin became dry, and the horny layer was not cast off but remained as a hard, thick layer (40, 55). The granular layer was more distinct than in normal rat skin (55).

III. STEROLS

Cholesterol is the principal sterol of the animal organism and is present in all living cells as free alcohol and esterified with fatty acids. In all tissues cholesterol is accompanied by a saturated derivative, dihydrocholesterol or β -cholestanol (pp. 323-24). In the routine cholesterol estimation methods, the sum of these two compounds is estimated. It is not known yet what percentage of the "cholesterol" in skin tissues is dihydrocholesterol. It is present in skin surface

doubled in pancreatectomized cats, in spite of a huge reduction in the skin's total fat content. In the same animals the cholesterol content of the liver is considerably decreased (21).

Available data are insufficient to calculate how the cholesterol is distributed between epidermis and dermis. In mouse epidermis 0.270 mg/mg protein-N was found (53, 44). This figure would indicate that the cholesterol/protein ratio is not

TABLE 2
CHOLESTEROL CONTENT OF SKIN, EXPRESSED IN MG. PER
100 GM. DRY SUBSTANCE TISSUE
(Average Values)

Species	Cholesterol (Mg. Per Cent)	Remarks
Adult rats (43)....	218	Subcutaneous fat removed before analysis
Adult rats (1)....	261	Specimens possibly contain subcutaneous fat
Adult dogs (28)....	394	Specimens possibly contain subcutaneous fat
Adult cats (21)....	350	Specimens possibly contain subcutaneous fat
Adult men (22)....	367	Subcutaneous fat removed before analysis

fats (p. 324). The cholesterol content of the skin, as compared with other tissues, is relatively high (42). Some of the values are given in Table 2. Unfortunately, in some cases, it is not clear whether subcutaneous fat was included or not.

There is a pronounced decline of cholesterol content in the skin tissue with age, as is best shown in the data of A. Meyer (22) (Table 3). Originally, it was thought that the diminution of cholesterol content with age was due to atrophy of the sebaceous glands (9). However, it was pointed out (22) that fetal skin, which has more than three times more cholesterol than adult skin, does not yet have well-developed sebaceous glands.

Changes in cholesterol content are entirely independent of changes in the amount of dermal depot fat. For instance, the cholesterol content of the skin is more than

much higher in the epidermis than in the corium. However, in human epidermis from palms and soles separated by acetic acid,

TABLE 3
TOTAL STEROL CONTENT OF SKIN EXPRESSED
IN MG. PER 100 GM. DRY SUBSTANCE (22)

	Sterols (Mg. Per Cent)
Fetal skin.....	1,203
Skin of newborn.....	647
Skin of children up to 14 years of age...	493
Skin of adults above 14 years of age....	367

Kooyman (17) found 0.74-1.13 per cent, or an average of 0.9 gm. cholesterol per 100 gm. dry weight, which appears to be two and a half to three times greater than the concentration in the total skin. It is to be hoped that, with satisfactory separation methods

available (51), direct comparative measurements of constituents in epidermis and corium in the same specimen of skin will soon be forthcoming.

The relation of free to esterified cholesterol in rat skin was estimated by Sturges *et al.* (43). While the total cholesterol content did not show regional variations, there were great regional differences in the free to total cholesterol ratio. Average values were 60 per cent free cholesterol in facial, 47 per cent in abdominal, and 35 per cent in dorsal skin. In rabbit skin Onizawa (26) found 81 per cent of the cholesterol nonesterified. He claimed that the skin, together with testi-

pointed out in 1929 (33). This was done in 1932 by Kooyman (17), who found Unna's claim to be true. He separated the epidermis of palms and soles from human cadavers by dilute acetic acid, scraped off the lower and the upper surfaces, and carried out lipid estimations separately in the total epidermis, in the lower (so-called "basal") layers, and in the upper (horny) layers. As Table 4 shows, Kooyman found only 1.8 per cent of the total cholesterol esterified in the basal layers, whereas in the horny layer 18.1 per cent were esterified. It should be emphasized that Kooyman's data were obtained from plantar and palmar epidermis,

TABLE 4*
LIPOID CONTENT OF EPIDERMIS OF PALMS AND SOLES (17)

Layer	Phospho- lipids	Total Cholesterol	Free Cholesterol (Per Cent of Total Cholesterol)	Phospho- lipids per Cholesterol	Total Fatty Acids
Total epidermis. . .	0.63	0.90	92.6	0.70	1.71
"Basal layers" . . .	2.62	1.56	98.2	1.8	3.94
Horny layer.	0.14	0.73	81.9	0.20	2.87

* Average lipid values in percentages of dry weight.

cles, muscles, brain, and lungs, belongs to the group of organs which have the lowest cholesterol ester content. No regular change of the free/total cholesterol ratio with age was found in human skin by A. Meyer (22). Her average ratio was 80 per cent free/total cholesterol.

Unna (46-49) was the first to claim that in man, *during keratinization of the epidermal cells, free cholesterol undergoes esterification* within the cornifying cells. This contention was based on the finding that a greater fraction of cholesterol is esterified in the horny layer than in the total epidermis. Unna's chemical (47, 48) and histochemical (49, 46) methods were rather crude and unreliable. Still, the difference between the esterification percentage in the horny layer and that in the total epidermis was so great that Unna's findings seemed to deserve re-examination with more reliable methods, as I

areas which are not supplied with sebaceous glands. Thus this function can be regarded purely as a function of epidermal cells.

Concerning the fate of cholesterol in the keratinization process, it was noted early that scales cast off in inflammatory diseases of the skin, collected mainly in cases of exfoliative dermatitides, are rich in cholesterol. In 1910 Salkowsky (34) isolated 0.15 gm. of pure cholesterol palmitate crystals from 4 gm. of scales of exfoliative dermatitis, that is, 4 per cent as calculated for the ester. Eckstein and Wile (14) found about 2 per cent cholesterol in both normal and exfoliative dermatitis scales. Santori and Caccarini (35) list similar values in scales from psoriatic, ichthyotic, and other pathological scales, namely, 0.8-3.8 per cent. Prokopschuk (30) found 0.5-2.3 per cent in psoriatic scales. Altogether, there is no clear-cut difference between the cholesterol content of

normal and that of pathological horny material. In the horny layer of palms and soles, for instance, Kooyman (17) found 0.73 per cent total cholesterol content, a value which is at the lower end of the pathological range but still within that range.

In the past a great point was made of the claim that horny material contains several times as much cholesterol as do tissues in general, and the epidermis in particular. This apparent "enrichment" or accumulation of cholesterol as keratinization of the epidermis proceeds led to all kinds of speculation, particularly to the assumption that

374). The cholesterol and cholesterol ester content of the fatty film on the skin surface in normal individuals and in skin diseases is discussed in chapter 13.

The occurrence of sterols other than cholesterol in the skin tissue of rats was reported recently by Baumann *et al.* (16). The greatest part of the so-called "fast-acting" sterols in skin tissues, which gave immediate color reaction with the Liebermann-Burckhardt procedure and which constituted nearly 30 per cent of the total sterol content, was identified by these authors largely as Δ^7 -cholestenol, a cholesterol derivative with

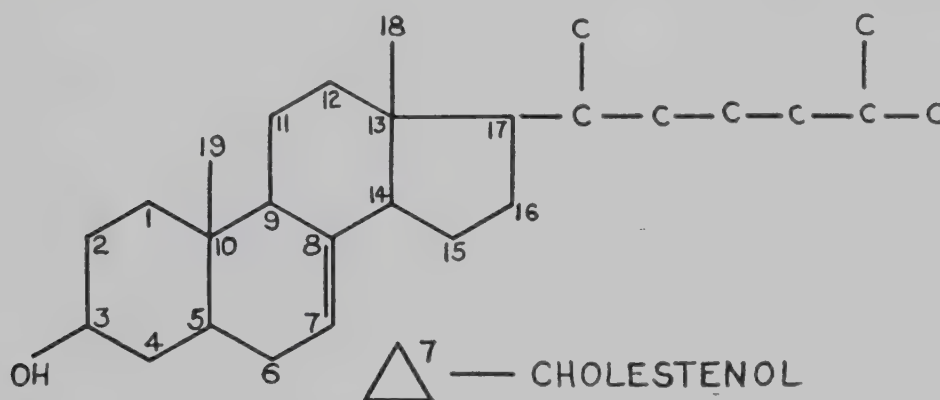


FIG. 1.—Structural formula of Δ^7 -cholestenol

the "formation" of cholesterol is an obligate factor in keratinization (33).

It is unnecessary to deal with details of this speculation because, actually, there is *no* enrichment in cholesterol in the horny layer. It looks as if the horny layer were rich in cholesterol only because of its extremely low water content. If the available data are calculated in percentages of dry weight, it appears that if anything happens to cholesterol during keratinization, it is rather a decrease than an increase in concentration. The data of Kooyman (17), for instance, indicate that, calculated for dry weight, the noncornified part of the epidermis contains 1.5 per cent cholesterol and the horny layer only half that much, namely, 0.73 per cent. It is quite obvious that the pseudo-increase in concentration of cholesterol in the horny layer is due exclusively to the dehydration which takes place during keratinization (p.

a double bond between C₇ and C₈ (Fig. 1) instead of between C₅ and C₆. Biologically active steroid hormones so far have not been shown to occur in skin tissue.

By far the most important finding in recent times has been the demonstration of the great activity of skin tissue in cholesterol synthesis. That the animal organism is able to synthesize cholesterol was first shown in balance experiments by Schönheimer (36). Bloch and Rittenberg (3), using deuterium-labeled acetate, and more recently Anker (2), using C¹⁴-labeled acetate, definitely established that cholesterol is synthesized by the liver from 2-carbon atom radicals. Chaikoff *et al.* (42), also using C¹⁴-labeled acetate, demonstrated that the skin tissue of the adult rat *in vitro* is as active in synthesizing cholesterol from acetate as is the liver; the skin of 1-day-old rats is two to two and a half times more active than the

liver. The two tissues which apparently are most concerned with cholesterol synthesis are the liver and the skin. In Chaikoff's experiments the skin probably accounted in large measure for the demonstrated cholesterol synthesis in eviscerated rats, in

view of the skin's bulk and high activity. Obviously, the biosynthesis of cholesterol is performed by the living cells of the epidermis, and this function is not connected with the keratinization process (pp. 374 and 487).

IV. PHOSPHOLIPIDS

Phospholipids are compound fat-soluble substances that contain in their molecule, in addition to fatty acids and glycerol, a nitrogenous base (such as choline or ethanol amine) and phosphoric acid. These vitally important compounds, with highly complex physiological and biochemical functions, are universally found in living cells, and their concentration in tissues is remarkably constant. The relation between phospholipids and mitochondria of the cytoplasm was recognized early (38). Mitochondria have physical and chemical properties similar to those of phospholipids, and there is close parallelism between the phospholipid and mitochondria content of tissues. The mitochondria, the site of complex enzymatic functions, play an important role in the physiological activities of all living cells.² In tissues with high activity, a high phospholipid content can be expected. Malignant tumors, for instance, contain three times as much phospholipid as do benign tumors. Or the phospholipid content of mammary glands in rabbits is doubled during lactation (7).

Phospholipids interact in the absorption of fats when the fatty acids of hydrolyzed fat are linked with the phosphoric acid-base complex. They are agents of oxidoreductive reactions by hydrogenation and dehydrogenation of the double bonds of their unsaturated fatty-acid components. They have a decisive role in the maintenance of the physical state of the protoplasm and particularly of the cell membrane (38, 39). A well-regulated balance exists between the concentration of phospholipids and that of

sterols. The biochemical activity of phospholipids is counteracted or regulated by the presence of cholesterol (8).

The phospholipid content of total mammalian skin in the adult is 0.7–0.8 per cent of the dry weight (21, 31). A remarkably large difference in the phospholipid content of the skin in young and adult animals was noted by Rewald and Schwieger (31). They found 1.18 per cent phospholipids in calves' skin and only 0.73 per cent in cows' skin as related to dry weight. These authors also claimed that the feeding of large doses of phospholipids (soybean oil) results in extraordinarily abundant hair growth in dogs, rabbits, and hogs.

Like young tissue, repair tissue is distinguished by a high phospholipid content. Taylor *et al.* (45) found that in healing skin wounds of rats the repair tissue makes about 30 per cent of the total lipids in the stage of most rapid healing, as compared with approximately 3 per cent in normal tissue.

In the epidermis, corresponding with its considerably higher cell content, the phospholipid content is higher than in the corium. In human palmar and plantar epidermis minus the horny layer, Kooyman (17) obtained 2.62 per cent phospholipids per dry weight (see Table 4, p. 486), which is more than three times the concentration of that of the total skin (45).

The basal cells of the epidermis, like any other actively proliferating cell type, are rich in mitochondria. They can be seen in the lower parts of the cytoplasm close to the corium. But as the cells move to higher strata, the mitochondria become scattered and diminish in number until they may be entirely absent in the stratum lucidum and horny layer (20). In perfect analogy to these

2. For results of modern research on mitochondria see Symposium: the structure and biochemistry of mitochondria, *J. Histochem. & Cytochem.*, 1:179–276, 1953.

microscopic findings are the results of chemical analyses. They show that, whereas in the lower layers of the epidermis the amount of phospholipids is considerable (2.62 per cent per dry weight), there is only 0.14 per cent per dry weight of tissue left when keratinization is completed (17) (see Table 4, p. 486).

As mentioned, the phospholipid/cholesterol ratio is regarded as a vitally important relation for the maintenance of the physiological colloidal state of the protoplasm. This relationship, as is to be expected in any

process connected with the death of cells, is greatly disturbed in the keratin layer. During keratinization only about half the cholesterol disappears (p. 487), whereas the phospholipids disappear almost completely: only about one-twentieth of the epidermal phospholipids is left in the horny cell. The ratio phospholipids/cholesterol thus decreases from 1.8 to 0.2 (see Table 4, p. 486). The disintegration of phospholipids during the keratinization process has also been indirectly demonstrated by following the fate of choline during keratinization (41).

V. CHOLINE

Choline is the nitrogenous base of lecithin, the most widely distributed phospholipid. As shown in Table 5, most of the choline (about 95 per cent) is present in bound form both in the epidermis and in the dermis of human skin as determined by the microbiological method. Apparently, choline is bound as a constituent of phospholipids. The heat-separated epidermis contains about six times more choline than does the heat-separated dermis. In scales, however, 60–80 per cent of the total choline is present in a free form, an indication that during keratinization phospholipids undergo considerable hydrolytic decomposition.

Another remarkable finding has been that the normal horny layer contains twenty times less choline than does the epidermis. Thus it seems that in the keratinization process choline is not only liberated from its phospholipid complex but also decomposed to a considerable degree.

Most work on choline in my laboratory (41) was done on pathological scales because of the easy availability of such material. Using the microbiological method, we found (Table 5) that in pathologically accelerated keratinization, as it occurs in postinflammatory scaling, in psoriasis, and in exfoliative dermatitis, decomposition of choline is less complete than if the keratinization process is physiological. In comparing our choline values with our clinical data, we came to the conclusion that the more the

keratinization is accelerated, the more choline is left in the scales.

These results were confirmed recently by Ottenstein *et al.* (27). These authors, working with both the microbiological and the chemical choline estimation methods, found,

TABLE 5
FREE AND TOTAL CHOLINE CONTENT OF
CUTANEOUS ELEMENTS (41)
(Mg/Gm Wet Weight)

	Free	Total	Per Cent Free
Epidermis, human cadaver, abdominal skin. . . .	0.067	1.2	5.6
Corium, human cadaver, abdominal skin.	0.009	0.18	5.0
Normal (scraped) scales.	0.051
Scales (postinflammatory, after sunburn).	0.022	.036	61.1
Psoriasis scales.175	.244	71.7
Exfoliative dermatitis scales (psoriatic).	0.376	0.455	82.7

in addition, that choline is closely bound to keratin in the horny layer and that the hydrolytic liberation of choline from phospholipids during keratinization is a function of the pH.

The destruction of phospholipids during keratinization involves liberation of free fatty acids, which may be responsible for the esterification of cholesterol in the horny

layer (p. 486) and for the esterification of wax alcohols (19). There is also a possibility that the large amount of free fatty acids on

the surface (p. 313) stem—at least in part—from the hydrolytic decomposition of phospholipids.

BIBLIOGRAPHY

- ABELIN, I. Chemical changes in the skin in experimental hyperthyroidism, *Biochem. J.*, **34**:229–33, 1940.
- ANKER, H. S. Some aspects of the metabolism of pyruvic acid in the intact animal, *J. Biol. Chem.*, **176**:1337–51, 1948.
- BLOCH, K. The metabolism of acetic acid in animal tissues, *Physiol. Rev.*, **27**:574–620, 1947.
- BLOOR, W. R. Distribution of unsaturated fatty acids in tissues. I. Beef heart muscle, *J. Biol. Chem.*, **68**:33–56, 1926.
- . Distribution of unsaturated fatty acids in tissues. II. Voluntary muscle of beef, *ibid.*, **72**:327–43, 1927.
- . Distribution of unsaturated fatty acids in tissues. III. Vital organs of beef, *ibid.*, **80**:443–54, 1928.
- . Phospholipid content and physiological activity of tissues, *Proc. Soc. Exper. Biol. & Med.*, **27**:294, 1930.
- BLOOR, W. R., and SNIDER, R. Phospholipid content and activity in muscle, *J. Biol. Chem.*, **107**:459–70, 1934.
- BÜRGER, M., and SCHLOMKA, G. Beiträge zur physiologischen Chemie des Alterns der Gewebe. IV. Untersuchungen an der menschlichen Haut, *Ztschr. f. d. ges. exper. Med.*, **63**:105–16, 1928.
- CARRIÉ, C. Untersuchungen über die chemischen Substanzen der Haut. I. Methode zur Bestimmung chemischer Substanzen auf der Haut, *Arch. f. Dermat. u. Syph.*, **173**:604–6, 1936.
- CARRIÉ, C., and HEEMEYER, R. Untersuchungen über die chemischen Substanzen der Haut. II. Über das Vorkommen von Kochsalz und Cholesterin auf der Haut, *Arch. f. Dermat. u. Syph.*, **173**:606–11, 1936.
- CARRIÉ, C., and KOENIG, R. Untersuchungen über die chemischen Substanzen der Haut. III. Über den Zucker-gehalt auf der Haut bei Normalen und Diabetikern, *Arch. f. Dermat. u. Syph.*, **173**:611–14, 1936.
- COSTELLO, C. J.; CARRUTHERS, C.; KAMEN, M. D.; and SIMOES, R. L. Uptake of radio-phosphorus in phospholipid fraction of mouse epidermis in methylcholanthrene carcinogenesis, *Cancer Research*, **7**:642–46, 1947.
- ECKSTEIN, H. C., and WILE, U. J. The cholesterol and phospholipid content of the cutaneous epithelium of man, *J. Biol. Chem.*, **69**:181–86, 1926.
- HALDI, J.; GIDDINGS, G.; and WYNN, W. Dietary control of the water content of the skin of the albino rat, *Am. J. Physiol.*, **135**:392–97, 1941–42.
- IDLER, D. R., and BAUMANN, C. A. Skin sterols. II. Isolation of Δ^7 -cholestenol, *J. Biol. Chem.*, **195**:623–28, 1952.
- KOONYMAN, D. J. Lipids of the skin. Some changes in the lipids of the epidermis during the process of keratinization, *Arch. Dermat. & Syph.*, **25**:444–50, 1932.
- . Lipids of the skin. Influence of some factors on the albino rat, *ibid.*, **29**:342–50, 1934.
- KOPPENHOEFFER, R. M. A review of the role of lipids in the manufacture of leather, *J. Am. Leather Chemists A.*, **37**:118–31, 1942.
- LUDFORD, R. J. Cell organs during keratinization in normal and malignant growth, *Quart. J. Micr. Sc.*, **69**:27–57, 1924.
- MATTHEWS, V. J.; NEWTON, J. K.; and BLOOR, W. R. Skin lipids in experimental diabetes, *J. Biol. Chem.*, **108**:145–51, 1935.
- MEYER, A. Vergleichende Gesamtsterin- und Sterinesterbestimmungen der Haut des wachsenden und erwachsenen Organismus, *Ztschr. f. Kinderh.*, **50**:596–607, 1931.
- MOHS, P. Welche Sterine sind im Wollfett enthalten? *Fette u. Seifen*, **45**:152–54, 1938.
- MOORE, P. R., and BAUMANN, C. A. Skin sterols. I. Colorimetric determination of cholesterol and other sterols in skin, *J. Biol. Chem.*, **195**:615–21, 1952.
- NADEL, A. Chemische Studien an der menschlichen Haut. II. Untersuchungen an

- der normalen und pathologischen Menschenhaut, Arch. f. Dermat. u. Syph., **165**: 507-24, 1932.
26. ONIZAWA, J. Studies on the behavior of cholesterol within the animal body. II. The content of free and ester cholesterol in various tissues of normal rabbits, J. Biochem., **10**:409-11, 1929.
 27. OTTENSTEIN, B.; BONCODDO, N.; WALKER, A.; and THURMON, F. M. Experiments on the choline content of the skin and sebum, J. Invest. Dermat., **19**:105-8, 1952.
 28. PARHON, C. I., and WERNER, G. Sur la teneur en cholestérol de la bile, de la peau et des organes, chez les animaux soumis au traitement thyroïdien, parathyroïdien, ou à ces deux traitements combinés, Compt. rend. Soc. de biol., **107**:401, 1931.
 29. ———. Teneur en eau, cholestérol, calcium et potassium de la peau, cholestérol du cristallin et calcium de l'humeur aqueuse, chez les animaux thyroparathyroïdectomisés, *ibid.*, **108**:989-91, 1931.
 30. PROKOPTSCHUK, A., and KIRSON, M. Cholesterin bei Haut- und venerischen Krankheiten, Arch. f. Dermat. u. Syph., **174**:90-95, 1936.
 31. REWALD, B., and SCHWIEGER, A. Über den Phosphatidgehalt der Haut, Biochem. Ztschr., **259**:180-81, 1933.
 32. ROTHMAN, S. Abnormalities in the chemical composition of the skin surface film in psoriasis, Arch. Dermat. & Syph., **62**:814-19, 1950.
 33. ROTHMAN, S., and SCHAAF, F. Chemie der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1**:2:161-377. Berlin: J. Springer, 1929.
 34. SALKOWSKY, E. Über das Vorkommen von Cholesterinestern in der menschlichen Epidermis und die Reaktionen derselben, Biochem. Ztschr., **23**:361, 1910. Quoted by ROTHMAN and SCHAAF (33).
 35. SANTORI, G., and CECCARINI, F. Ricerche sui rapporti tra psoriasi e ricambio lipidico. II. Sul contenuto colesterinico della cute e delle squame nella psoriasi, Gior. ital. di dermat. e sif., **88**:205-18, 1947.
 36. SCHÖNHEIMER, R. Versuch einer Sterinbilanz an der legenden Henne, Ztschr. f. physiol. Chem., **185**:119-22, 1929.
 37. SCHÖNHEIMER, R.; BEHRING, H. VON; and HUMMEL, R. Über die Bedeutung gesättigter Sterine im Organismus. IV. Untersuchung der Sterine aus verschiedenen Organen auf ihren Gehalt an gesättigten Sterinen, Ztschr. f. physiol. Chem., **192**:93-111, 1930.
 38. SINCLAIR, R. G. The physiology of the phospholipids, Physiol. Rev., **14**:351-403, 1934.
 39. ———. Fat metabolism, Ann. Rev. Biochem., **6**:245-68, 1937.
 40. SMEDLEY-MACLEAN, I., and HUME, E. M. Fat deficiency disease of rats. The storage of fat in the fat-starved rat, Biochem. J., **35**:990-95, 1941.
 41. SNIDER, B. L.; GOTTSCHALK, H. R.; and ROTHMAN, S. The fate of choline in normal and pathologic keratinization, J. Invest. Dermat., **13**:323-24, 1949.
 42. SRERE, P. A.; CHAIKOFF, I. L.; TREITMAN, S. S.; and BURSTEIN, L. S. The extrahepatic synthesis of cholesterol, J. Biol. Chem., **182**:629-34, 1950.
 43. STURGES, S., and KNUDSON, A. Application of the Schoenheimer-Sperry method to the determination of cholesterol and cholesterol esters in tissues, J. Biol. Chem., **126**:543-50, 1938.
 44. SUNTZEFF, V.; CARRUTHERS, C.; and COWDRY, E. V. The role of epidermal lipids in the response of immature mice to the carcinogenic action of methylcholanthrene, Acta Unio internat. contra cancrum, **6**:386-88, 1948.
 45. TAYLOR, J. D.; PAUL, H. E.; and PAUL, M. F. Biochemistry of wound healing. III. Total lipid, phospholipid, and cholesterol content of skin and repair tissue of skin wounds, Arch. Biochem., **17**:421-28, 1948.
 46. UNNA, P. G. Histochemie der Haut. Leipzig and Vienna: F. Deuticke, 1928.
 47. UNNA, P. G., and GOLODETZ, L. Die Hautfette, Biochem. Ztschr., **20**:469-502, 1909.
 48. ———. Die Cholesterinester der Hornschicht, *ibid.*, **25**:425-26, 1910.
 49. UNNA, P. G., and SCHUMACHER, J. Lebensvorgänge in der Haut der Menschen und Tiere. Leipzig and Vienna: F. Deuticke, 1925.
 50. URBACH, E. Beiträge zu einer physiologischen und pathologischen Chemie der Haut. II. Der Wasser-, Kochsalz-, Reststickstoff-, und Fettgehalt der Haut in der Norm und

- unter pathologischen Verhältnissen, Arch. f. Dermat. u. Syph., **156**:73-101, 1928.
51. VAN SCOTT, E. J. Mechanical separation of the epidermis from the corium, J. Invest. Dermat., **18**:377-79, 1952.
52. VLÈS, F., and UGO, A. Sur l'excitation de la fluorescence du cholestérol et de la peau, Compt. rend. Soc. de biol., **123**:226-31, 1936.
53. WICKS, L. F., and SUNTZEFF, V. Reduction of total lipid-protein nitrogen ratio of mouse epidermis by single application of methylcholanthrene, J. Nat. Cancer Inst., **3**:221-26, 1942.
54. ———. Changes in epidermal cholesterol during methylcholanthrene carcinogenesis in mice, Cancer Research, **5**:464-68, 1945.
55. WILLIAMSON, R. A note on the epidermis of the rat on fat-free diet, Biochem. J., **35**:1003-5, 1941.
56. WYNN, W., and HALDI, J. Water and fat content of albino rat on high fat diet, further observations, Am. J. Physiol., **142**:508-11, 1944.

CHAPTER 21

Water and Electrolytes

I. WATER CONTENT OF THE SKIN	493
A. Water Content of Total Skin	493
B. Water Content of the Epidermis	494
C. Distribution of Water in the Corium	495
D. Influence of Age	495
II. THE MOBILE WATER AND SALT DEPOT OF THE SKIN	496
III. ELECTROLYTE CONTENT OF THE SKIN	500
A. Chlorides	500
B. Sodium	500
C. Sodium and Chloride Relationship in Total Skin	500
D. Potassium	501
E. Sodium and Potassium in the Epidermis	501
IV. MAGNESIUM AND CALCIUM	502
A. Magnesium	502
B. Calcium	503
V. THE POTASSIUM/CALCIUM RATIO	504
VI. TOTAL BASE CONTENT OF THE SKIN	504
VII. MICROINCINERATION	506
VIII. WATER AND MINERALS IN TRAUMATIZED AND DISEASED SKIN	508
A. Water, Sodium, and Chloride	508
B. Potassium and Calcium	509
C. Magnesium	510

I. WATER CONTENT OF THE SKIN

A. WATER CONTENT OF TOTAL SKIN

THE determination of the water content of the skin with no adherent subcutaneous fat yields rather constant values under standardized technics and experimental conditions. In a particular animal species, specimens must be taken from a specified site from animals of the same age group and sex; the animals must be kept on a standard diet; the skin must be thoroughly homogenized; and the values must be related to the weight of fat-free skin (94, 2, 17, 13).

The extraction of fat before the estimation of water content is necessary because of the highly variable amounts and distribution of fat in the corium (94, 2) (p. 483) and also because of the difficulty in removing subcutaneous fat completely (17). If concentrations of electrolytes are to be related to total weight or to water content, it is more logical to express values in terms of fat-free skin, because electrolytes are biologically active only when dissolved in water. The admixture of more or less fat, which is not

miscible with water, is accidental (75). The work of Eichelberger *et al.* on the skin of dogs (17, 20) and man (22) clearly showed that reproducible results can be obtained only if water and electrolyte values are expressed in their relation to the weight of fat-free skin.

In order to obtain the water content and express it in terms of fat-free tissue, the skin, as removed from the body, is dried to constant weight and then extracted with dry ethyl ether. Thus only the "free" fat is removed. Complete removal of all fatty constituents from the corium requires acidification prior to extraction (63), apparently because of the presence of lipoproteins, which have to be split in order to remove all the "fat" (63). Because the fat obtained after acid hydrolysis contains the intracellular and vitally important lipids, it is probably more correct for analytical purposes to extract the skin at body pH. Other methodological problems in the estimation of the water content of the total skin were dealt with by F. Herrmann (40), by Haldi *et al.* (102), and by others.

The skin is not an organ particularly rich in water. In man the weight of the skin is 16–18 per cent of the total body weight (84), and its water content is 18–20 per cent of the total water content of the body. Skeletal muscles have more water absolutely and relatively. They constitute 41–42 per cent of the total body weight and contain about half the total body water (84). Lepore (50) found that the skin has the lowest amount of water per unit weight among the soft tissues, and some older data indicate the same situation (79).

Under carefully controlled experimental conditions, Eichelberger (17) found the water content of fat-free shaved skin of adult normal dogs to be 70.83 per cent \pm 2.01. In man, fresh surgical skin specimens from breast and from legs yielded 69.0–74.0 per cent water in fat-free skin, with an average value of 71.77 per cent (22). Older data on the water content of the skin in man were estimated without previous fat extraction and showed much greater variations, such

as 56.7–71.4 per cent (3), 58.2–67.8 (69), 63.8–70.6 (68). Some of the average values in animal skin, with fat not extracted, are: dog, 63.86 per cent (50); ox, 61 per cent (63); calf, 63 per cent (63); rat, 60 per cent (2). In general, values obtained without fat extraction are closer to 60 than to 70 per cent. The inverse relation of water and fat content in rats kept on different diets was shown by Haldi *et al.* (33, 34, 35, 102) (see Table 1, chap. 20).

Probably, the variable fat content is only one of the reasons for variations in water content. Another reason is the inhomogeneity of the skin with variable proportions of the different elements with different water content. The main reason, however, I believe, is the great ease with which water is shifted to and from the corium according to the needs of the general water metabolism (see p. 496). This variable water content can be eliminated somewhat if, prior to the determination, food and fluid intake as well as environmental temperature and humidity are kept constant for several days and if the excision is done after fasting for a definite period of time.

B. WATER CONTENT OF THE EPIDERMIS

The published values on the water content of the epidermis were obtained from heat-separated epidermis, a method which does not seem suitable for estimation of water because of the evaporation during separation (17). Still, at least for mouse epidermis, Suntzeff and Carruthers (89) found that the water loss in heat separation is negligible. Values obtained by scraping off the epidermis from stretched skin and those obtained by heat separation were practically identical: 59.5–62.6 and 59.5–61.5 per cent, respectively, in unshaved skin. By relating the concentration of single constituents to the wet weight in the heat-separated epidermis, these authors obtained as constant and reliable results as by relating concentration to nucleoprotein-phosphorus content.

In 15 specimens of human heat-separated epidermis, Zheutlin and Fox (103) found 59.4–69.9 per cent water, with an average

value of 64.5 per cent. Thus the water content of the human epidermis happens to be in the same range as that of total skin containing fat. However, one has to consider the presence in the epidermis of the horny layer, which is extremely poor in water. The water content of the rete alone is probably considerably higher. The great variations found by Zheutlin and Fox (103) might be caused partly by different relative thicknesses of the horny layer.

C. DISTRIBUTION OF WATER IN THE CORIUM

It was thought that the papillary layer contains considerably more water than the reticular layer (79). However, if one calculates the respective values on a fat-free basis, this difference becomes smaller or is reversed, as shown in Table 1.

ways associated with a constant amount of water and electrolytes. If one assumes that the fibrous material of all connective tissues, whether dense or loose, corresponds in this respect with that of tendons and if one determines the collagen-nitrogen content, one can calculate for any connective tissue how the water is partitioned between fibrous and nonfibrous phases. On this basis Eichelberger (15) calculated the distribution of water in the corium. According to this calculation, out of the average 715 gm. of water in 1 kg. of fat-free human corium, 390 gm. of water are associated with fibers and 325 gm. with nonfibrous proteins.

D. INFLUENCE OF AGE

In the newborn the water content of the skin is much higher than in the adult organism. For rats this was shown by Lowrey

TABLE 1
WATER CONTENT OF UPPER AND LOWER CORIUM IN HUMAN SKIN (94, 68)

Subject	Water in Whole Skin (Per Cent)	Fat Content (Per Cent)	Fat-free Solids (Per Cent)	Water in Fat-free Skin (Per Cent)
<i>Not specified (94):</i>				
Epidermis+papillary layer....	63.20	1.52	35.28	64.2
Reticular layer.....	54.63	17.19	28.18	66.0
<i>Male, 21 years (68):</i>				
Epidermis+papillary layer....	69.52	0.58	29.90	69.9
Reticular layer.....	57.59	10.12	32.09	64.3
<i>Male, 28 years (68):</i>				
Epidermis+papillary layer....	68.81	0.36	30.83	69.1
Reticular layer.....	63.35	1.64	35.01	64.4

The data of McLaughlin and Theis (63) on the skin of oxen also reflect differences in fat content rather than in water content. These authors studied a specimen with a total water content of 61 per cent. The corium was divided so that the upper layer represented 20 per cent, the middle layer 50 per cent, and the lower layer 30 per cent of the total weight. The water content was 74.35 per cent in the upper, 61 per cent in the middle, and only 29.78 per cent in the lower layer.

Earlier work on tendons (65) indicated that the fibrous material in tendons is al-

(59). In human infants the water content of the skin is 81-82 per cent (95, 94). From fetal life on to the age of sixty there is a progressive decline in water content (4). According to the data of A. Meyer (67), the average values of the water content of the total skin are 80 per cent in the fetus, 68.4 per cent in the newborn, and 62.1 per cent in adolescents and adults. In senescence, however, there is a considerable increase again (95, 94, 4). It is remarkable that senile skin, which appears to be "dry," has a relatively high water content. Of course, what is usually called "dry skin" refers to the ap-

pearance of the surface and has nothing to do with the water content. As a matter of fact, conditions with so-called "dry skin"

TABLE 2*

WATER CONTENT OF THE SKIN OF ALBINO RATS ON VARIOUS DIETS (102)

	Stock Diet	High-Carbohydrate Diet	High-Fat Diet
Males.....	64.7	60.8	62.3
Females.....	58.1	54.6	57.5

* Values are expressed in percentages of fat-free skin.

were often reported to contain more water than normal, e.g., skin in senile degeneration, ichthyotic skin (94), and skin in different atrophic states (p. 509).

A striking sex difference in the water content of the skin of albino rats was demonstrated by Haldi *et al.* (34). Independent of the diet, female skin has a significantly lower water (and protein) content than the skin of males. Although one part of this sex difference is due to higher fat content in the skin of females, differences remain when the values are calculated for fat-free skins (Table 2).

II. THE MOBILE WATER AND SALT DEPOT OF THE SKIN

A great mass of water is accumulated in the corium. Roughly, in a man weighing 65 kg. the skin contains 7.5 kg. of water. This amount can change considerably according

TABLE 3*

AVAILABLE AND INTRACELLULAR WATER IN ORGANS OF NORMAL DOGS (24)

	Available Water	Intracellular Water	Solids
Skin.....	0.627	0.105	0.268
Rectus abdominis muscle.....	.123	.563	.275
Liver.....	.340	.401	.259
Spleen.....	0.410	0.366	0.224

* Values are expressed in cubic centimeters per gram.

total extracellular fluid volume of the body, including plasma and interstitial fluid, has been called "available water" and can be measured by the thiocyanate dilution method. About 75 per cent of the total available water is found in the musculature and in the skin. The skin contains about four times as much available water per unit weight as muscles do (Table 3). Because the total mass of muscles is about four times as much as that of the skin, the absolute amount of available water in muscles and skin is in the same range (24) (Table 4). However, if it comes to either hydration or severe dehydration, the role of the skin is disproportionate-

to the needs of the organism. As contrasted with other organs, such shifts in water content can occur in the corium without any appreciable functional disturbance (79). Excess water is stored there, and in severe dehydration the largest water loss is borne by the large connective-tissue mass of the skin. Older data on this function of the skin from the schools of Magnus in Utrecht and of E. P. Pick in Vienna (79) have been confirmed in newer contributions, particularly in the excellent work of Flemister (24).

The greatest part of the water in the skin is in the interstitial fluid, which can be regarded as an ultrafiltrate of plasma. The

TABLE 4

AVAILABLE WATER IN ORGANS OF RABBIT, 3 KG. (24)

	Skin	Muscle	Liver	Other Organs (Calc.)	Total
Available water (cc.)....	258	219	53	100	630
Percentage of total available water..	41.0	34.8	8.4	15.8	100

ly high both in receiving excess and in delivering required available water (Tables 5 and 6). This disproportionality becomes obvious only in excessive hydration and de-

hydration. If dehydration is moderate, all tissues lose water roughly proportionally to their available water content (84, 24). Whether the skin has a special position because its connective-tissue mass is more continuous than that of muscle or for some other reason is not known.

In an experiment by Eichelberger *et al.* (17), the water retention of the skin in pregnancy was shown by an increase in water content of from 70.83 to 75.21 per cent in the fat-free tissue. In dogs made hypertensive by the Goldblatt technic, an increase in

the water content from 70.83 to 74.71 per cent was observed. Even in these hypertensive dogs with so much water in their skins, the brunt of fluid excess created by intravenous saline injections was carried by the corium. After such injections (170 cc/kg body weight) the water content of the skin rose from 74-75 per cent to 82 per cent, a percentage increase much greater than in any other organ (17).

If water is withdrawn by energetic dehydration, the loss in water is borne almost exclusively by the skin, no matter whether

TABLE 5
AVAILABLE WATER INCREASED BY 34 PER CENT THROUGH INTRA-
PERITONEAL FLUID INFUSION IN RABBIT, 3 KG. (24)

	Gain per Gm. Tissue (Cc.)	Gain of Normal Available Water (Per Cent)	Gain by Whole Organ (Cc.)	Total Gain Accepted (Per Cent)
Skin.....	0.225*	36	92.7	43.0
Semitendinous muscle.....	.030	24	47.0	21.8
Rectus abdominis muscle...	.050	31	15.6	7.2
Liver.....	.100	29
Heart ventricle.....	.070	21
Spleen.....	0.140	34	60.7	28.0
Other organs (calc.).....
Total.....	216.0	100.0

* Boldface figures in Tables 5 and 6, not used in the original contribution, indicate the overwhelming participation of the skin.

TABLE 6
50 PER CENT OF AVAILABLE WATER WITHDRAWN THROUGH INTRAPERI-
TONEAL INJECTION OF HYPERTONIC GLUCOSE IN SALINE
IN RABBIT, 3 KG. (24)

	Loss per Gm. Tissue (Cc.)	Loss of Normal Available Water (Per Cent)	Loss by Whole Organ (Cc.)	Contribu- tion to Total Loss (Per Cent)
Skin.....	0.400	64	165.0	53.0
Semitendinous muscle.....	.035	28	65.8	21.0
Rectus abdominis muscle...	.050	31	15.6	5.0
Liver.....	.100	29
Heart ventricle.....	0.068	20
Spleen.....	65.6	21.0
Other organs (calc.).....
Total.....	312.0	100.0

dehydration is produced by fluid withdrawal (82, 94, 13), by diuretics (82, 86), or by loss of blood (83). Cold environmental temperature also effects a considerable loss of water from the skin without damage (37).

De Boer (13) experimented with chronic and acute dehydration on dogs. In chronic dehydration caused by starvation and thirst the skin showed the greatest percentage decrease in water (5 per cent), while the decrease in muscle and blood was only 1 per cent. During recovery, the muscles regained their original water content much faster than did the skin. In acute dehydration produced by hemorrhage or by injection of hypertonic sucrose, there was 7 per cent loss of water in the skin and none from the musculature; the blood gained 2 per cent water at the same time. Thus it appears again that the skin "can be classed as a reservoir capable of taking care of a large part of any excess fluid added to the body" (17) and also "acts as an emergency regulator of body water" when water is withdrawn (13).

In overloading the organism with sodium chloride, again, the skin takes up relatively most of the excess (79, 32). This fact, recognized long ago, was confirmed in modern experiments by the use of isotopes. The data of Fox *et al.* (27) reveal that if mice are infused with a solution of Na^{24}Cl , the skin accumulates two to three times more radioactivity, expressed in percentages of total radioactivity injected per gram of tissue, than do skeletal muscles. Remarkably, this ability of the skin is retained also in burned limbs but not in limbs which were made ischemic by a tourniquet. In the latter case the concentration of Na^{24} in skin and muscles is about the same (27). Earlier it was found that inflamed skin lost some of its ability to hold salts (93, 94, 85).

The good salt-holding capacity of the connective tissue of the skin can be contrasted with the rapid excretion of intravenously infused sodium chloride through sweat glands. Radioactive sodium can be demonstrated in sweat in less than 60 seconds after injection (5).

If sodium chloride is withdrawn, again the mobile depot of the skin is primarily utilized to maintain the salt content of the blood and internal organs (79). This was demonstrated in low-salt diets for chloride by Urbach (96) and for sodium by Dörffel (14). I pointed out (77) that if it is true that low-salt diets influence beneficially cutaneous tuberculosis but not tuberculosis of other organs, this might be because, among all organs, the skin undergoes the most drastic changes in its chemical composition when salt is withdrawn.

The skin is particularly well adapted to function as a mobile depot for water and sodium chloride mainly because its water content and salt concentration can be changed independently of each other. For instance, in experiments of De Boer (13) with acutely dehydrated dogs, the chloride concentration in the remaining water of the skin increased by 60 per cent and in chronically dehydrated dogs by 23 per cent. In contrast, in muscles chronic dehydration caused not an increase, but a loss, of chloride of 22 per cent. This gain by the skin of 23 per cent and loss by muscle of 22 per cent shows that, while the skin is able to hold salt independently of its water content, such independent regulation is not possible in the musculature.

It might appear surprising that abrupt changes in water and salt intake are not immediately followed by adjustment on the part of the kidneys. However, when extensive adjustment becomes necessary, the change in water balance does not closely follow the change in extracellular fluid volume (38). If large amounts of salt are given—for instance, 20 gm. per day—there is a large gain in body weight caused by water retention, but the gain in sodium is much greater. In one experiment of Urbach (94) the cutaneous gain in water was 2 per cent, the cutaneous gain in chloride 15 per cent. In loading the body with sodium chloride, there is transfer of water from the intra- to the extracellular compartments, in an attempt to establish osmotic equilibrium. But even this water transfer does not pre-

vent changes in total electrolyte concentration; there is an increase in the sodium concentration of the interstitial fluid (75). That there is a lag in the renal removal of a large load of sodium placed in the extracellular fluid compartment was shown by Stewart and Rourke (87). The load of disbalance is primarily carried by the skin.

It is most probable that water retention and dehydration or salt retention and salt deprivation are reflected most prominently in the skin not only under experimental conditions but also as they occur in human pathology, irrespective of the causation. Little experimentation has been done in this field. But it was claimed that the water uptake by the skin proper is quite prominent in kidney insufficiency (81) ("pre-edema"), in obesity (94), in acromegaly (94), and in nutritional deficiencies (35). In experimental hyperthyroidism of rats Abelin (1) found abnormally high water and chloride values in the skin. A decrease in water content was found in diabetes insipidus (93) and in parathyroid insufficiency (44). Nothing is known about the relative participation of the skin in the retention of water and salt under the influence of adrenocortical hormones.¹

In what physical form the surplus water is stored in the skin is not well understood, although physicists and chemists of the leather industry have studied in detail the different forms of water-binding by hides. If fresh hides are pressed intensely, a large amount of water can be squeezed out. But a fraction of water is held so tightly that it seems to be chemically bound to the tissue. Accordingly, one speaks of "free" and "bound" water. These expressions have been used rather vaguely, and it appears that the relative amount of bound water is different according to the definition or, rather, to the method by which it is esti-

mated (21). In many instances bound water in the corium and in other hydrophilic colloids has been defined as that part of water which will not dissolve water-soluble nonelectrolytes. Eilers and Labout (21) presented evidence that the "nonsolvent" or bound water consists of two parts, viz., (1) approximately 20 per cent of the total water is actually bound to the colloid and will not dissolve even the smallest molecules of nonelectrolytes; and (2) water is present in tiny cavities of the colloidal substance which are too small to permit the penetration of larger molecules but which will be able to dissolve smaller molecules. If saccharose is used as the nonelectrolyte the solubility of which is being measured, this fraction of bound water also amounts to approximately 20 per cent of the total water in fresh hides (21). The remaining 60 per cent is "free" water, which surrounds either single amino acid chains or the whole micelle. The connective tissue of the skin is able to take up large amounts of "free" water. Whether this is a property more of collagen fibers or of ground substance is at present a moot question.

The theories of water-binding by fibrous proteins are manifold. One of the most important theories is that binding is caused by electrostatic forces, generated by ionizable groups of the proteins on the dipoles of water. However, the effect of the pH on the binding of water is not so great as it should be if this were true (21), so that electrostatic forces do not account for all phenomena.²

Early data (44, 76) seemed to indicate that the ability of the corium to swell in acids is increased after thyroidectomy. However, just the reverse, Abelin (1) found that the swelling ability of the corium, both in acids and in alkalies, is tremendously increased after administration of toxic doses of thyroid.

1. Recently a careful study was published by Cole (9) on water and electrolyte changes in the skin after adrenalectomy in the rat.

2. The water uptake of collagen is discussed on p. 405; the water uptake of ground substance, on pp. 420 ff.

III. ELECTROLYTE CONTENT OF THE SKIN

The weight relationship of corium to epidermis is about 9:1 (20, 17). The great mass of the skin represents acellular connective tissue; and analyses of electrolytes in total skin reflect this situation.

A. CHLORIDES

The skin is by far the richest organ in chlorides. In older publications the chloride concentration of human skin was estimated to lie between 0.2 and 0.3 per cent (79). Taking the average value of Urbach (94) of 0.26 per cent, this amounts to 73.2 mEq/kg wet tissue or, calculated for its concentration in water, to 97.6 mEq/l of water.³ In recent work, in which data are expressed in terms of fat-free skin, the values found ranged between 73.5 and 85.5 mEq., with an average of 79.9 ± 4.8 mEq/kg in human skin (22). In the dog even higher values were found: 106 mEq/kg whole skin (99); 113 mEq/l of tissue water (36); and 86.7 ± 2.5 mEq/kg of fat-free skin (17).

Comparing these values with the chloride content of muscles and viscera, one finds that they are twice as high or higher in the skin than in other organs (83, 50, 10). The data of Eichelberger *et al.* (17, 8), obtained from analysis of fat-free tissue, show that the chloride content of skin is four times that of skeletal muscle. The difference is striking also if the chloride concentration is calculated per unit of water. The chloride concentration of tissue water in the skin far exceeds that in other organs (75). Chloride is the predominating anion of interstitial fluid, and the high chloride values in skin probably can be explained on the basis that the total skin is relatively rich in interstitial fluid and relatively poor in cells.⁴

3. Values related to volume of water imply that the space occupied by proteins is neglected (29).

4. While it is safe to state that the main extracellular anion is Cl^- and the main intracellular anions are HCO_3^- , organic HPO_4^- , SO_4^- , and protein (29), recent work strongly suggests that chloride is also present in considerable quantities outside the extracellular fluid space (25), i.e., in the cells.

B. SODIUM

Like the chloride concentration, the concentration of sodium is unusually high in the total skin as compared with other organs. Sodium is the main extracellular cation, and its amount in the skin is high, probably because of its great extracellular mass. In man's fat-free skin the sodium concentration was found to be 80–112 mEq., or an average of 93.0 ± 8 mEq/kg wet weight (22). In the dog the corresponding values were 90.9–106.3 mEq., or an average of 96.5 ± 4.2 (17). When fat was not removed, a value of 60.8 mEq/total tissue weight was obtained (38).

C. SODIUM AND CHLORIDE RELATIONSHIP IN TOTAL SKIN

There is an excess of sodium equivalents over chloride equivalents in blood plasma, the Na/Cl ratio being 1.38:1. In most interstitial fluids this ratio is slightly smaller, namely, 1.26:1 (17). The reason for this inequality is this: When fluid passes through a membrane which is impermeable to proteins, the Donnan effect can be observed; this is a state of ionic equilibrium set up between two sides of a membrane in a solution of electrolytes with one ion on one side to which the membrane is impermeable. In the case of plasma versus interstitial fluid, the "membrane" is impermeable to anionic proteins. To preserve cation-anion equivalence with electrical neutrality on both sides, there must be an adjustment of concentrations of diffusible ions. Because the anionic proteins do not pass the membrane, there must be a reduction of cations or/and an increase in anions in the interstitial fluid. Most available data have confirmed the original assumption that the relationship of concentrations in plasma and interstitial fluid conforms with the Donnan equilibrium.

From calculations by Peters (75) on the basis of Harrison, Darrow, and Yannet's (38) data, it would appear that there is an excess of sodium over chloride in the skin as in all other tissues, the ratio Na:Cl being

1.34:1. However, Eichelberger (17) calculated a ratio of 1.11:1 for dogs' skin, which would indicate that, relative to sodium, there is more chloride in the skin than in other organs. Therefore, she concluded that, because the composition of extracellular fluid must be the same everywhere, since it is a plasma ultrafiltrate, some of the chloride in the skin is intracellular (see also Manery *et al.* [64, 66]).

D. POTASSIUM

The main cations inside the cell are potassium and magnesium. In plasma and interstitial fluids the concentration of potassium is low, and the ratio of potassium to sodium is about 1:28. Potassium, like sodium, exists in a freely diffusible state in the plasma, and its concentration in normal tissue fluid as well as in edema fluid conforms with the Donnan equilibrium (25).

Since most of the skin is acellular connective tissue, one would expect a potassium to sodium relationship in total skin similar to that of interstitial fluids. This, however, is not the case. The relative amount of potassium is much higher, possibly because of the high potassium content of the epidermis and other cellular elements of the skin, such as follicular epithelium, sweat-gland cells, sebaceous-gland cells, endothelial cells, and fibroblasts. The data of Eichelberger (22) reveal a potassium to sodium relation of 1:5.65 in fat-free human skin and 1:4.3 in fat-free shaved dogs' skin. According to older data, the relative amount of potassium is even more than that: from three independent publications (3, 14, 10) a potassium to sodium ratio of 1:2.6 can be calculated for total human skin. There are probably two main reasons for these discrepancies: older methods of sodium determination were not reliable, and the early determinations were not done on fat-free skin.

The range of potassium values match well throughout the literature: Expressed in milliequivalents per 100 gm. dry human skin, the average potassium concentrations were given as 6.6 (70), 6.1 (3), 6.6 (14), and 6.3 (10) as compared with the more recently

obtained value of 5.83 in fat-free dry skin (22). In the estimations of Cornbleet *et al.* (10) the normal values were found to be in the range of 6.15–6.7 mEq/100 gm dry substance.

While the potassium content of the skin is much higher than one would expect on the basis of its great interstitial mass, it is still much lower than in most other soft tissues. In puppies, Hamilton and Schwartz (36), expressing the potassium values in milliequivalents per liter of tissue water, found the following concentrations: liver, 111; muscle, 93; brain, 89; kidney, 78; skin, 29. In dogs the potassium concentration of soft tissues, as determined and expressed on a fat-free basis by Eichelberger *et al.*, were (a) skeletal muscle, 97.1 ± 7.5 mEq. (8); (b) liver, 73.2 ± 6.3 mEq. (8); (c) brain, 95.6 ± 4.7 mEq. (19); (d) kidney, 58.3 ± 4.0 mEq. (16); and (e) skin, 22.46 ± 2.7 mEq. (17).

E. SODIUM AND POTASSIUM IN THE EPIDERMIS

Estimations of sodium and potassium in heat-separated human epidermis was made by Suntzeff and Carruthers (88) and by Zheutlin and Fox (103). There is fairly good agreement for values obtained in human epidermis, as shown in Table 7, although the ranges are rather wide. This table shows that, in comparison with total skin, there is a tremendous accumulation of potassium in the human epidermis, absolutely as well as relative to sodium. The concentration of potassium is close to twice that of sodium.

Eichelberger (20) has obtained quite different values in dogs' heat-separated epidermis, absolutely as well as in the potassium to sodium relationship. Her average values were 31.02 mEq. sodium and 12.2 mEq. potassium per 100 gm. epidermal solids. The two sets of data cannot be reconciled. Moreover, from data on the potassium content of whole skin, of corium alone, and of potassium associated with fiber solids, Eichelberger (20) calculated values which are approximately the same as she obtained by direct determination. In spite of the high

potassium values shown in Table 7, the potassium to sodium ratio in the epidermis is lower than in other cellular organs; in other words, the sodium content is relatively high (103). Whether all the surplus sodium is to be placed in the extracellular spaces (intercellular canals) of the rete and in the intraepidermal portions of the sweat

same range, the corresponding sodium values of the epidermis of the leg are far below those in the breast epidermis. The average values are: for the leg, 94 mEq. sodium and for the breast, 143.25 mEq. There is a possibility that the sodium content parallels the amount of intercellular fluid space in the epidermis, i.e., its succulence; and one may

TABLE 7
SODIUM AND POTASSIUM CONTENT OF THE EPIDERMIS

AUTHOR	SPECIES	SODIUM (mEq/Kg FRESH EPIDERMIS)		POTASSIUM (mEq/Kg FRESH EPIDERMIS)	
		Range	Average	Range	Average
Zheutlin and Fox (103).....	Human	29-62	46.14	73.6-96.0	80.46
Suntzeff and Carruthers (88)...	Human	44.3-62.5	54	70.2-96.2	82
Carruthers and Suntzeff (7)...	Mouse	64.8-79.5	73	86.7-89.5	88

ducts, as considered by Zheutlin and Fox (103), or whether reservations must be made concerning the exclusively extracellular site of sodium (29) is not known at the present time.

A peculiar regional difference can be recognized from the data of Zheutlin and Fox (103). Whereas the potassium value in the epidermis of breast (12 samples) and leg (3 samples) related to dry weight are in the

assume that the epidermis of the leg is less succulent than the epidermis of other parts, in spite of a greater water content of the corium on the leg. The data of Zheutlin and Fox indicate that the concentration of the sum of sodium and potassium ions in human epidermis (126 mEq/kg wet weight) is close to that in red blood corpuscles (136 mEq/kg wet weight) (103).

IV. MAGNESIUM AND CALCIUM

A. MAGNESIUM

Magnesium is regarded as a strictly intracellular cation. As far as the skin is concerned, analyses of the separated layers do not clearly reflect this situation. From Suntzeff and Carruthers' data one can calculate an average magnesium concentration of 2.48 mEq. in 100 gm. fresh human epidermis, while the value for fat-free corium solids in the dog, determined by Eichelberger (20), has been 2.03 ± 0.43 mEq., or, calculated for wet weight, approximately 0.61 mEq. In other words, if one assumes that the coriums of man and of dogs have a similar mineral content, it appears that the magnesium concentration of the epidermis is about four times greater than that of the

corium. The difference should be, however, more pronounced if the magnesium content were more or less proportionate to the cell content of tissues. Actually, microincineration observations indicate an overwhelming accumulation of magnesium in the epidermis (23). Harrison *et al.* (38) calculate 56 mEq. magnesium per liter of intracellular water in dogs' skin, and this relatively high value again suggests high intracellular concentration. Obviously, a great amount of further work is required to establish the range and averages in the separated layers of the skin in different species. The new separation of Van Scott (97) based on simple stretching and the greatly simplified methods of estimation, particularly flame spectro-

photometry, will probably greatly facilitate such work.

According to older data (3, 14), total human skin contains about 2.4 mEq. magnesium per 100 gm. dry weight, and it was stated that the range is about the same as that of calcium.

B. CALCIUM

Older analyses of Brown (3) on the calcium content of the whole human skin agree well with newer analyses. Values of other earlier authors (70, 14) are far too low. It appears from Table 8 that the average calcium content of human and dog skin is about 2 mEq/100 gm dry weight. That of rabbit skin is higher.

The most important physiological factor influencing the calcium content of the skin is age. With progressing age, the calcium content increases (3, 70). The difference is so great that it could be clearly demonstrated even by the rather coarse qualitative microincineration method (41). There is a most pronounced increase in calcium content in the mouse epidermis with age; per 100 gm. tissue, 18 mg. in the newborn, 44 mg. in the two-to-three-month-old adult, and 54 mg. in the twelve-to-thirteen-month-old senescent animal (11).

The calcium content of heat-separated epidermis and corium was estimated in dog skin by Eichelberger (20). In 100 gm. dry fat-free solids the corium contained 2.38 mEq. calcium (determined); the epidermis, 0.88 mEq. (estimated). In other words, this analysis revealed 2.7 times more calcium in the corium than in the epidermis. Because Suntzeff and Carruthers' (88) absolute epidermal values are much higher (calculated for dry epidermis, 2.2 mEq.) than those of Eichelberger, this great difference requires further study. Still, a relatively low calcium content of the epidermis was postulated for a long time on the basis of histochemical findings (100, 31, 57). Also, data on other organs indicate that parenchymatous tissues contain less calcium than connective tissues.

It is known that part of the intracellular

calcium is bound to protein and is thought to be an integral part of the cell periphery or cell "membrane" (39). It is also held responsible for the coherence of cells to each other (49). Lansing (49) estimated the ultrafilterable free and nonultrafilterable protein-bound fractions of the calcium in normal mouse epidermis and found that only 38.4 per cent of the total calcium is in a free state.

A high calcium content of the granular layer was found by Lansing (p. 375).

Early histochemical findings and chemical analyses indicate that the horny layer

TABLE 8
AVERAGE CALCIUM CONTENT OF TOTAL SKIN

Author	Species	Calcium (mEq/ 100 Gm Dry Tissue)	Remarks
Brown (3).....	Human	2.30	
Cornbleet <i>et al.</i> (10).....	Human	2.14	
Eichelberger (22)	Human	1.80	mEq/100 gm fat-free solids
Brown (3).....	Dog	2.10	
Eichelberger (17)	Dog	2.06	mEq/100 gm fat-free solids
Eichelberger (20)	Dog	2.22	mEq/100 gm fat-free solids
Brown (3).....	Rabbit	3.70	
Klauder and Brown (42)...	Rabbit	3.16	

and hair are richer in calcium than is the noncornified rete (79). The sebaceous glands, allegedly having a high calcium content (12, 30), may contribute to the impregnation of the horny layer with calcium salts.

Nothing definite is known about the distribution, linkages, and physiological role of calcium in the corium, except that it represents a vital constituent of the cementing substance of endothelial cells (see p. 64). However, it is possible that the corium plays the role of a mobile calcium depot; McLaughlin and Theis (63) found a 50 per cent decrease in the calcium content of the skin in cows subsequent to exhaustive milk-

ing. There is some indirect evidence that calcium may be bound to connective-tissue fibers in a nonionized form (18).

An increase in the calcium content of arterial elastin with age was demonstrated by Lansing *et al.* (48).

V. THE POTASSIUM/CALCIUM RATIO

It was claimed that different types of diets may change the ratio of potassium to calcium in the skin. The specific claim was that if rabbits are fed green food containing a basic ash, the ratio is lower than if the animals are fed with oats containing an acid ash. It was also claimed that if the potassium to calcium ratio becomes greater than normal, the irritability of the skin in response to inflammatory stimuli increases (79). This contention could not be well substantiated (79), as can be best seen from the extensive work of Klauder and Brown (42, 43). Although in the majority of cases these authors actually found that the skin is less irritable, the higher its calcium and the

lower its potassium content, this was not the general rule. Eichelberger (20) pointed out that the potassium to calcium ratios in epidermis and corium are so different (12.0 and 3.4, respectively) that if the whole skin ratio is estimated, which is normally 3.8, ratio changes in the epidermis may be completely masked because of the greater mass of the corium. If the relation of irritability to mineral content should be investigated in the future, separate estimations in the separated layers will be necessary. Even then, difficulties of interpretation may arise because of the presence of sweat constituents in the epidermis and also because of the changing relationship of ionic to bound calcium.

VI. TOTAL BASE CONTENT OF THE SKIN

In three early publications (3, 14, 10) there is good agreement of data on concentrations of sodium, potassium, calcium, and magnesium in normal human skin. Newer data were obtained after fat extraction and, therefore, cannot be directly compared with

termination of sodium were not quite reliable. I have calculated the available data in simple weight percentages and in equivalent weight percentages. The data thus obtained are shown in Tables 9, 10, 11, and 12.

TABLE 9
CONCENTRATION OF BASES EXPRESSED IN MG/100 GM DRY HUMAN SKIN

Author	Na	K	Ca	Mg	Total	Remarks
Brown (3).....	360	239	46	30	675	Mg was not determined; total base is calculated on the assumption that concentration of Mg equals that of Ca
Dörffel (14).....	361	260	27	29	677	
Cornbleet (10).....	350	247	43	683	
Eichelberger (22).....	757	228	38	18	1,041	In dry fat-free skin

the old data. Still there remains a great discrepancy in sodium values, even if 30 per cent of the dry skin is taken as free fat. Thereby the total base content, as well as the percentage distribution of the cations, becomes uncertain and requires re-examination. Possibly the older methods for de-

As compared with total skin, the total base content of the epidermis is much higher than, and the distribution of the single cations in the epidermis is very different from, that in whole skin. Calculation from Suntzeff and Carruthers' data reveals that the total base content of the dry epidermis is

1,431 mg/100 gm dry tissue or 147.1 mMol/100 gm dry tissue (88). Table 13 shows the percentage distribution of the single cations in human epidermis.

In summary, the total base content is one and a half to two times as high in the epi-

dermis as it is in the corium (1,431 mg. versus 1,041 mg.). While in the corium the predominant cation is sodium and the amount of potassium is relatively small, the sodium to potassium relationship is just about the reverse in the epidermis.

TABLE 10
CONCENTRATION OF BASES EXPRESSED IN MEQ/100 GM DRY HUMAN SKIN

Author	Na	K	Ca	Mg	Total	Remarks
Brown (3).....	15.6	6.1	2.3	2.46	26.46	See remark in Table 9 See remark in Table 9
Dörffel (14).....	15.7	6.6	1.36	2.38	26.04	
Cornbleet (10).....	15.2	6.3	2.14	26.78	
Eichelberger (22) ..	32.9	5.83	1.90	1.50	42.88	

TABLE 11*
PERCENTAGE DISTRIBUTION OF BASES WHEN CONCENTRATION IS
EXPRESSED IN MG/100 GM DRY WEIGHT

Author	Na	K	Ca	Mg	Remarks
Brown (3).....	53.3	35.4	6.81	4.4	See remark in Table 9 See remark in Table 9
Dörffel (14).....	53.3	38.4	4.0	4.3	
Cornbleet (10).....	51.3	36.2	6.7	
Eichelberger (22).....	62.7	21.9	3.7	1.7	

* The figures indicate percentage of total base.

TABLE 12*
PERCENTAGE DISTRIBUTION OF BASES WHEN CONCENTRATION IS
EXPRESSED IN MEQ/100 GM DRY WEIGHT

Author	Na	K	Ca	Mg	Remarks
Brown (3).....	59	23	9	9	See remark in Table 9 See remark in Table 9
Dörffel (14).....	60	25	6	9	
Cornbleet (10).....	57	24	8	8	
Eichelberger (22).....	77	14	4	3	

* The figures indicate percentage of total base.

TABLE 13
PERCENTAGE DISTRIBUTION OF CATIONS IN TOTAL BASE
(DRY HUMAN EPIDERMIS) (88)

	Na	K	Ca	Mg
Expressed in weight per cent per total base.....	25.6	67.5	3.1	3.8
Expressed in molarity per cent per total base.....	36.7	55.8	2.5	5.0

VII. MICROINCINERATION

Although the microincineration method has yielded many details on the topographic distribution of inorganic material in the skin on a microscopic scale (30, 41, 46, 23), the over-all results have been rather meager, mainly because only calcium and perhaps

The spodograms indicate a greater accumulation of ash in the epidermis than in the corium. The residue of elastic and collagenous fibers shows little structural detail (30, 46), but calcium can be localized along elastic fibers, basement membranes, and

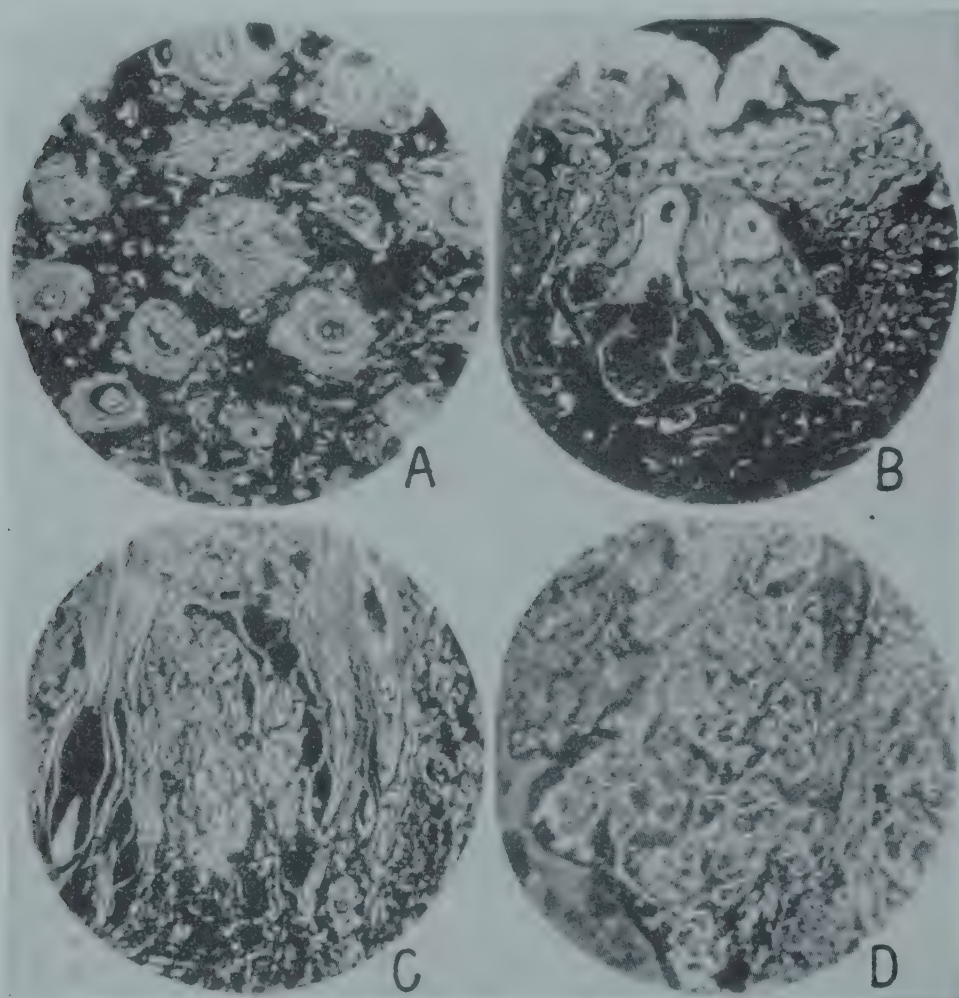


FIG. 1.—Photomicrographs of microincinerated sections of skin. *A*, hair follicles of scalp; *B*, sebaceous glands from elbow; *C*, eccrine glands from scalp; *D*, apocrine glands from axilla. From Gans (30). (Reproduced by permission of Springer-Verlag.)

magnesium ashes can be identified with fair certainty. The structural details of cross-sections of the skin are well preserved after ashing. Cell walls and nuclei are recognized by accumulations of ash, and appendages are well outlined because of the relatively high ash content of basement membranes (Fig. 1).

glass membranes of hairs (30, 46). Interestingly, some of the minerals disappear when sections are treated with fat solvents prior to ashing (46) (Fig. 2).

The ash content of the epidermis in inflamed skin was found to be greater than normal, particularly the calcium ash. Such findings were reported in psoriasis, "ecze-

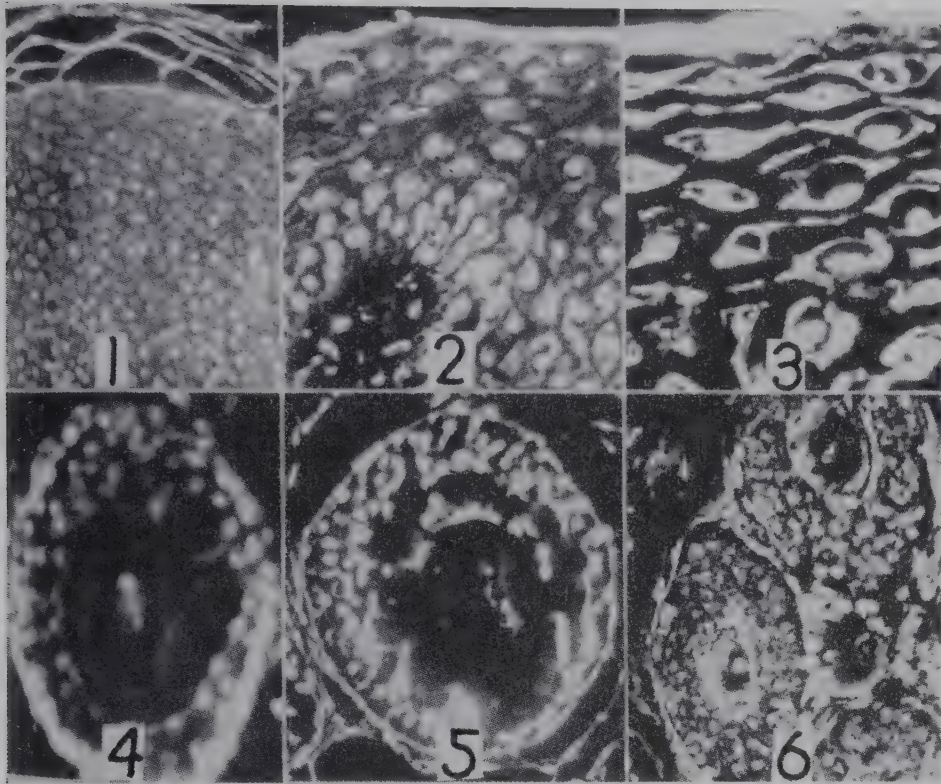


FIG. 2.—Photomicrographs of microincinerated sections of skin. The sections shown in the upper row were taken from the skin of the shoulder of a patient with exfoliative dermatitis (fixation with absolute alcohol and formaldehyde). In 1 is shown an unextracted section; in 2 a section extracted with cold ether; and in 3 a section extracted with hot acetone. The sections shown in the lower row were taken from the skin of a monkey and cut parallel to the surface, showing hair follicles. In 4 is shown an unextracted section; in 5 a section extracted with chloroform; and in 6 a section extracted with 1 per cent acetic acid. From Kooyman (46). (Reproduced by permission of the author and the American Medical Association.)

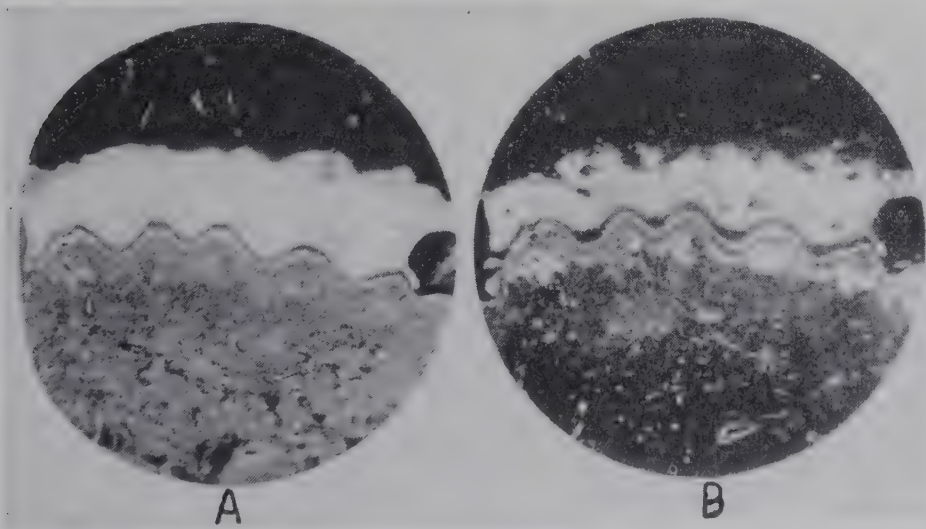


FIG. 3.—Skin of sole. *A*, total ash; *B*, calcium ash. The layers of the stratum corneum are well separated. Stratum pre-eleidinicum—narrow white wavy band. Stratum lucidum—wide black wavy band. Stratum posteledinicum—gray caps on the peaks of the waves. From Gans (30). (Reproduced by permission of Springer-Verlag.)

ma," ultraviolet dermatitis (30), contact dermatitis, lichen planus, exfoliative dermatitis, and pemphigus (41).

A peculiar periodicity of ash deposits in keratinizing epithelium was noted by Gans (30) (Fig. 3).

VIII. WATER AND MINERALS IN TRAUMATIZED AND DISEASED SKIN

In traumatized tissue there is an increase in water content, a huge increase in sodium, and considerable decrease in potassium concentration (26). These changes are caused by (1) accumulation of extracellular fluid in the traumatized tissue, (2) loss of potassium from the injured cells, and (3) exchange of sodium from the extracellular compartment for the intracellular potassium extruded (26). In damaged tissue the antisodium barrier of cells is abolished (27). Together with the increase in sodium content, there is an increase in concentration of chloride (26). In shock caused by tissue damage, hypotonicity and excess potassium in the extracellular fluid permit water and excess potassium to enter even uninjured cells (26).

This state of water-electrolyte disbalance in shock can develop by purely cutaneous traumatization, e.g., in burns. However, the electrolyte changes seem to be less pronounced in the traumatized skin tissue itself than in traumatized muscle (26). Loss of potassium from the skin might be less marked in traumatized skin tissue because its original potassium content is low. The less pronounced increase in sodium content might reflect the ability of the skin to mobilize or retain water and salt more independently than can other organs. In spite of the smaller gain in sodium in traumatized skin, this gain always exceeds what would correspond to the accumulation of extracellular fluid.

A. WATER, SODIUM, AND CHLORIDE

In *acute inflammatory processes* the water content of the skin is always greatly increased, and the chloride content is even more elevated (94, 71). This is obviously caused by accumulation of extracellular fluid or, in other words, by edema formation following vascular damage. The increase in water content strictly parallels the severity

of inflammation in the skin: there is a linear relationship between the percentage increase of water content and the logarithm of the concentration of the irritant (101).

Epidermis and corium participate in the uptake of abnormally large amounts of water, probably according to the intensity of damage to these layers. In eczematous reactions water uptake by both layers is obvious from microscopic observation. In erysipelas (94), where there is little epidermal change, probably only the water content of the corium changes. The most remarkable findings in this field have been data on an abnormally high water content in normal (uninvolved) skin of patients with "eczema" (atopic dermatitis?) (94), possibly in analogy to the condition of traumatic shock. Some data indicate that when the acute inflammation subsides, the water content becomes abnormally low (71, 72).

High water and chloride values were obtained in lesions of seborrheic dermatitis, lichen planus, pityriasis rosea (71) and psoriasis (94, 71) in the acute and subacute phases, and exfoliative dermatitis (94).

An increase of water content and a shift of electrolytes in inflamed skin are very much less pronounced in starvation and in water and/or salt withdrawal (85). This phenomenon was thought to explain the beneficial effects of hunger, thirst, and low-salt diets in different skin diseases.

In the twenties the generally accepted view was that in *pemphigus* there is considerable retention of water and chlorides in the skin (79). It was shown, however, relatively early, that this is not a constant phenomenon and, therefore, that water and salt retention cannot be a pathogenetic factor in pemphigus (68). The dramatic therapeutic results with ACTH and cortisone, both of which promote salt retention, make it even more clear that the old view that salt reten-

tion is a pathogenetic factor in this disease was erroneous. Still the findings of Urbach (94, 93) on the great avidity of the skin in pemphigus in retaining excess dietary sodium chloride are remarkable and worthy of rechecking. Urbach (93) interpreted the salt retention not as a primary manifestation of the disease but as a defense mechanism. If this is true, one may assume that in this disease ACTH and cortisone may act—partially at least—by promoting salt retention.

In more modern, extensive, and careful studies on pemphigus, Lever *et al.* (92, 55, 56, 51, 52, 54, 53) found a profound disturbance in water and salt metabolism, comparable in many respects to that in adrenal insufficiency (92). The plasma, blood, and interstitial fluid volumes were greatly increased in all patients with generalized skin lesions. In the blood serum the sodium concentration was particularly low, but also the chloride and calcium values were subnormal (92, 56). Lever also studied the electrolyte composition of blister fluid (55) and the proteins of blood plasma and blister fluid (50, 51, 53, 52) in this disease.

In acetic acid-separated epidermis of pemphigus patients Szodoray *et al.* (91) found abnormally low calcium values. This was thought to be the cause of the diminished adherence of rete cells in pemphigus.

Among *chronic inflammatory diseases*, lupus vulgaris was most extensively studied for the water and mineral changes in its lesions, mainly because of the observed beneficial effect of salt-poor diets. There is agreement that in lupus vulgaris lesions the water, chloride, and sodium content are abnormally high (14, 68). The calcium content is moderately increased (14). Chloride may be as high as 0.56 gm/100 gm dry tissue (68). Again, the salt content is relatively more elevated than the water content, a situation which in the old literature was called "dry retention." Salt-poor diets actually remedy these chemical anomalies (14, 96). One might think that the beneficial effect of vitamin D₂ administration, too, is based on changes in mineral composition in

the skin lesions. However, Van Scott's extensive experiments (98) in Dr. Eichelberger's laboratory and in mine did not show any changes in the mineral composition of the normal skin of normal dogs after huge doses of vitamin D₂ had been given over a long period of time.

Abnormally high water and chloride values were also found in chronic lichenified lesions.

Otherwise, in lesions of chronic non-exudative erythemato-squamous eruptions the changes in water and mineral content are very much less conspicuous than in acute inflammation. In psoriasis lesions the water content is only moderately increased, and the chloride content is usually unchanged (68) when the process is chronic.

As in senile atrophic skin (p. 496), a high water content was also found in other atrophic states, such as acrodermatitis chronica atrophicans (94). In atrophic lesions of scleroderma the increase in chloride content was more conspicuous than the increase in water content (94).

B. POTASSIUM AND CALCIUM

In connection with the alleged significance of the potassium to calcium ratio for the irritability of the skin (p. 504), several authors tried to prove that the concentration of potassium is increased and the concentration of calcium decreased in acute inflammatory processes. This claim never was well substantiated. In intense acute inflammatory processes the potassium content seems to decrease rather than to increase (72), and this finding corresponds more satisfactorily with the modern views on events in acute tissue damage. A decrease in calcium content was reported in almost all inflammatory diseases (42, 71, 72, 74, 14), but the data are far from being consistent.

In chronic lesions of psoriasis, if all layers of the lesion were analyzed *in toto*, the potassium content was found to be greatly increased—from an average of 259.5 mg. to an average of 491.5 mg. in 100 gm. dry tissue (14). This change might be caused simply by the acanthosis: the cellular content of

psoriasis lesions is much greater than normal, and epithelial cells are rich in potassium.

There is good reason to believe that in diverse degenerative processes of connective tissue, as in senile degeneration (47), there is an accumulation of calcium. The most extreme example is that of pseudoxanthoma elasticum (58). Both by observation of spodograms and by quantitative chemical analysis, Lobitz and Osterberg (58) demonstrated a huge amount of calcium in these tissues. As compared with normal skin, the amount of calcium is increased up to 5.6 fold. While in the normal skin calcium contributes 4.6 per cent to the total ash, this percentage in pseudoxanthoma elasticum lesions is 33 per cent.

In the uninvolved skin of a scleroderma patient I found calcium values entirely within the normal range (78). Whereas previously it was thought that patients with scleroderma retain injected calcium for abnormally long periods (80), my more recent experiments have not borne out this view. In diffuse generalized scleroderma the calcium balance is normal, and excess calcium ad-

ministered by mouth or by injection is excreted in a normal fashion (78). Thus the reason why scleroderma is so often associated with calcinosis remains obscure. It requires further studies to find out which kinds of connective-tissue degeneration do lead to calcium depositions in the skin and which ones do not.

C. MAGNESIUM

In a series of publications Engman and MacCardle (23, 60, 61, 62), on the basis of microincineration findings and spark-spectrographic analyses, reported on a conspicuous magnesium deficiency in cutaneous lesions of neurodermatitis and also on subnormal magnesium values in the normal uninvolved skin in this disease. No absolute values were given, but the comparative figures were as follows: normal skin of healthy individuals, 0.059–0.300; uninvolved skin of neurodermatitis patients, 0.025–0.108; and neurodermatitis lesions, 0.010–0.092. It was tentatively assumed that neurodermatitis is a magnesium-deficiency disease; but this interesting angle was not followed up any further.

BIBLIOGRAPHY

- ABELIN, I. Chemical changes in the skin in experimental hyperthyroidism, *Biochem. J.*, **34**:229–33, 1940.
- BORNSTEIN, A., and KERB, J. Chlor- und Wasserstoffwechsel bei der Sublimatvergiftung. Zugleich Bemerkung über die Technik der Veraschung tierischer Organe zur Chlorbestimmung, *Biochem. Ztschr.*, **126**:120–27, 1941.
- BROWN, H. The mineral content of human, dog, and rabbit skin, *J. Biol. Chem.*, **68**:729–36, 1926.
- BÜRGER, M., and SCHLOMKA, G. Beiträge zur physiologischen Chemie des Alterns der Gewebe. IV. Untersuchungen an der menschlichen Haut, *Ztschr. f. exper. Med.*, **63**:105–16, 1928.
- BURCH, G.; REASE, P.; and CRONVICH, J. Rate of sodium turnover in normal subjects and in patients with congestive heart failure, *J. Lab. & Clin. Med.*, **32**:1169–91, 1947.
- BURCH, G. E.; THREEFOOT, S. A.; and RAY, C. T. Rate of turnover and biologic decay of chloride and chloride space in the dog determined with the longlife isotope, Cl^{36} , *J. Lab. & Clin. Med.*, **35**:331–47, 1950.
- CARRUTHERS, C., and SUNTZEFF, V. Chemical studies on the mode of action of methylcholanthrene on mouse epidermis, *Cancer Research*, **3**:744–48, 1943.
- CHILDS, A., and EICHELBERGER, L. The distribution of water and electrolytes between blood, fluids and skeletal muscle in pregnant dogs, *Am. J. Physiol.*, **137**:384–91, 1942.
- COLE, D. F. The effects of adrenalectomy on the electrolyte composition of the skin of the rat, *Acta endocrinol.*, **11**:9–20, 1952.
- CORNBLEET, T.; INGRAHAM, R. C.; and SCHORR, H. C. Calcium, potassium, and sodium metabolism and the skin; the use of potassium chloride in certain allergic

- dermatoses, *Arch. Dermat. & Syph.*, **46**:833-40, 1942.
11. COWDRY, E. V.; CARRUTHERS, C.; and SUNTZEFF, V. Influence of age on the copper and zinc content in the epidermis of mice undergoing carcinogenesis with methylcholanthrene and a note on the role of the calcium, *J. Nat. Cancer Inst.*, **8**:209-13, 1948.
 12. DÄHN, W. Über die Kalzium- und Kaliumverteilung in der normalen Haut, *Dermat. Wchnschr.*, **82**:425-33, 1926.
 13. DE BOER, BENJAMIN. Alterations in the blood, and in the water, fat, and chloride content of tissues during dehydration in the dog. Ph.D. thesis, University of Missouri, 1942.
 14. DÖRFFEL, J. Klinische, experimentelle, und chemische Studien über den Einfluss der Ernährung auf Entzündungsvorgänge in der gesunden und kranken Haut, *Arch. f. Dermat. u. Syph.*, **162**:621-81, 1930-31.
 15. EICHELBERGER, L. Personal communication.
 16. EICHELBERGER, L., and BIBLER, W. G. Water and electrolyte content of normal and hydronephrotic kidneys, *J. Biol. Chem.*, **132**:645-56, 1940.
 17. EICHELBERGER, L.; EISELE, C. W.; and WERTZLER, D. The distribution of water, nitrogen, and electrolytes in the skin, *J. Biol. Chem.*, **151**:177-89, 1943.
 18. EICHELBERGER, L., and MCLEAN, F. C. The distribution of calcium and magnesium between the cells and the extracellular fluids of skeletal muscle and liver in dogs, *J. Biol. Chem.*, **142**:467-76, 1942.
 19. EICHELBERGER, L., and RICHTER, R. B. Water, nitrogen, and electrolyte concentration in brain, *J. Biol. Chem.*, **154**:21-29, 1944.
 20. EICHELBERGER, L., and ROMA, M. Electrolyte and nitrogen distribution in whole fat free skin and heat-separated corium and epidermis, *J. Invest. Dermat.*, **12**:125-38, 1949.
 21. EILERS, H., and LABOUT, J. W. A. The "non-solvent" water in hides. In: *Fibrous proteins: proceedings of a symposium held at the University of Leeds in May, 1946*, pp. 30-37. Bradford: Society of Dyers and Colourists, 1946.
 22. EISELE, C. W., and EICHELBERGER, L. Water, electrolyte and nitrogen content of human skin, *Proc. Soc. Exper. Biol. & Med.*, **58**:97-100, 1945.
 23. ENGMAN, M. F., and MACCARDLE, R. C. Histochemical study of neurodermatitis; preliminary report: microincineration and spectrographic analysis, *Arch. Dermat. & Syph.*, **42**:109-11, 1940.
 24. FLEMISTER, L. J. Distribution of available water in the animal body, *Am. J. Physiol.*, **135**:430-38, 1941-42.
 25. FOLK, B. P.; ZIERLES, K. L.; and LILIEN-THAL, J. L., JR. Distribution of potassium and sodium between serum and certain extracellular fluids in man, *Am. J. Physiol.*, **153**:381-85, 1948.
 26. FOX, C. L., and BAER, H. Redistribution of potassium, sodium, and water in burns and trauma and its relation to the phenomena of shock, *Am. J. Physiol.*, **151**:155-67, 1947.
 27. FOX, C. L., and KESTON, A. S. The mechanism of shock from burns and trauma traced with radiosodium, *Surg., Gynec. & Obst.*, **80**:561-67, 1945.
 28. FRIEBOES, W. Beiträge zur Anatomie und Biologie der Haut. X. Die biologischen Funktionen der menschlichen Epidermis, *Arch. f. Dermat. u. Syph.*, **140**:467-80, 1922.
 29. GAMBLE, J. L. Chemical anatomy, physiology, and pathology of extracellular fluid. Lecture syllabus, Harvard University, 1947.
 30. GANS, O. Zur Histo-Topochemie der gesunden und kranken Haut, *Arch. f. Dermat. u. Syph.*, **161**:607-46, 1930.
 31. GANS, O., and PAKHEISER, T. Über den Calciumgehalt der gesunden und kranken Haut, *Dermat. Wchnschr.*, **78**:249-60, 1924.
 32. GERÉB, S., and LÁSZLÓ, D. Über die Bedeutung der Haut für den Salz- und Wasserstoffwechsel, *Klin. Wchnschr.*, **1**:775-79, 1930.
 33. HALDI, J., and GIDDINGS, G. Water content of various organs of albino rat on high carbohydrate and high fat diet, *Am. J. Physiol.*, **128**:537-43, 1939-40.
 34. HALDI, J.; GIDDINGS, G.; and WYNN, W. Dietary control of the water content of the skin of the albino rat, *Am. J. Physiol.*, **135**:392-97, 1941-42.
 35. ———. The effect of vitamin B complex deficiency on the water content of the body

- and various organs of the albino rat, *ibid.*, **141**:83-87, 1944.
36. HAMILTON, B., and SCHWARTZ, R. The composition of tissues in dehydration, *J. Biol. Chem.*, **109**:745-53, 1935.
 37. HAMILTON, W. F., and BARBOUR, H. G. Heat regulations and water exchange; fate of fluid leaving blood in cold anhydremia, *Am. J. Physiol.*, **73**:321-23, 1925.
 38. HARRISON, H. E.; DARROW, D. C.; and YANNET, H. The total electrolyte content of animals and its probable relation to the distribution of body water, *J. Biol. Chem.*, **113**:515-29, 1936.
 39. HEILBRUN, L. V. An outline of general physiology. 2d ed. Philadelphia: W. B. Saunders Co., 1943.
 40. HERRMANN, F. Über die Wasserbindung in der Haut, *Ztschr. f. d. ges. exper. Med.*, **76**:780-91, 1931.
 41. ———. Erweiterung des Verfahrens des Schnittveraschung. Differenzierung der anorganischen Struktur gesunder und kranker Haut, *Ztschr. f. wissenschaft. Mikr.*, **52**:251-75, 1935.
 42. KLAUDER, J. V., and BROWN, H. Experimental studies in eczema. II. A correlation of the chemistry with the irritability of the skin of animals under normal and under experimentally induced conditions, *Arch. Dermat. & Syph.*, **15**:1-15, 1927.
 43. ———. Experimental studies in eczema. III. The role of the sympathetic nervous system and calcium-potassium ratio in altering cutaneous irritability in the rabbit, *ibid.*, **19**:52-65, 1929.
 44. KÖNIGSTEIN, H. Über Wasserverschiebung in der Haut unter physiologischen und pathologischen Bedingungen, *Arch. f. Dermat. u. Syph.*, **154**:352-67, 1928.
 45. ———. Physiologie der Haut. In: ARZT and ZIELER, Die Haut und Geschlechtskrankheiten, **1**:141-316. Vienna and Berlin: Urban & Schwarzenberg, 1934.
 46. KOOYMAN, D. J. State and localization of inorganic salts in the skin as revealed by extraction and microincineration, *Arch. Dermat. & Syph.*, **32**:394-403, 1935.
 47. LANSING, A. I. Calcium and growth in aging and cancer, *Science*, **106**:187-88, 1947.
 48. LANSING, A. I.; ALEX, M.; and ROSENTHAL, T. B. Calcium and elastin in human arteriosclerosis, *J. Gerontol.*, **5**:112-19, 1950.
 49. LANSING, A. I.; ROSENTHAL, T. B.; and AU, M. H. Ultrafilterable and non-ultrafilterable calcium in normal, hyperplastic epidermis and squamous cell carcinoma, *Arch. Biochem.*, **16**:361-65, 1948.
 50. LEPORE, M. J. Experimental edema produced by plasma protein depletion, *Arch. Int. Med.*, **50**:488-505, 1932.
 51. LEVER, W. F., *et al.* The proteins in pemphigus vulgaris. I. Electrophoretic analysis of the proteins in the blood serum of patients with pemphigus vulgaris, *J. Invest. Dermat.*, **14**:205-17, 1950.
 52. ———. The proteins in pemphigus vulgaris. II. Electrophoretic analysis of the proteins in the blister fluid of patients with pemphigus vulgaris, *ibid.*, pp. 219-25.
 53. LEVER, W. F.; HURLEY, N. A.; and BLANEY, A. E. The proteins in pemphigus vulgaris. IV. Determination of the plasma proteins by electrophoresis and chemical fractionation in patients with pemphigus under treatment with ACTH or cortisone, *J. Invest. Dermat.*, **19**:55-70, 1952.
 54. LEVER, W. F., and MACLEAN, J. G. The proteins in pemphigus vulgaris. III. The effect of infusions of human serum albumin on the proteins in the blood serum of patients with pemphigus vulgaris, *J. Invest. Dermat.*, **15**:215-22, 1950.
 55. LEVER, W. F., and TALBOTT, J. H. Electrolyte content of blister fluid in pemphigus, *J. Invest. Dermat.*, **5**:303-9, 1942.
 56. ———. Pemphigus. A further report on chemical studies of the blood serum and treatment with adrenocortical extract, dihydrotachysterol or vitamin D, *New England J. Med.*, **231**:44-51, 1944.
 57. LIEBER, G. D. Physikalisch-chemische Wirkung der Röntgenstrahlen, *Strahlentherapie*, **18**:536-44, 1924.
 58. LOBITZ, W. C., JR., and OSTERBERG, A. E. Pseudoxanthoma elasticum: microincineration, *J. Invest. Dermat.*, **15**:297-98, 1950.
 59. LOWREY, G. The growth of the dry substance in the albino rat, *Anat. Rec.*, **7**:143-68, 1913.
 60. MACCARDLE, R. C., and ENGMAN, M. F. Histology of neurodermatitis, *Arch. Dermat. & Syph.*, **44**:161-89, 1941.
 61. ———. Spectrographic analysis of neurodermatitis; human magnesium deficiency, *ibid.*, pp. 429-40.

62. ———. A new approach to the problem of disseminated neurodermatitis, *ibid.*, **46**: 337–47, 1942.
63. McLAUGHLIN, G. D., and THEIS, E. R. Notes on the animal skin composition, *J. Am. Leather Chemists A.*, **19**:421–41, 1924.
64. MANERY, J. F., and BALE, W. F. Penetration of radioactive sodium and phosphorus into extra- and intracellular phases of tissues, *Am. J. Physiol.*, **132**:215–31, 1941.
65. MANERY, J. F.; DANIELSON, I. S.; and HASTINGS, A. B. Connective tissue electrolytes, *J. Biol. Chem.*, **124**:359–75, 1938.
66. MANERY, J. F., and HAEGE, L. The extent to which radioactive chloride penetrates tissues, and its significance, *J. Biol. Chem.*, **134**:83–93, 1941.
67. MEYER, A. Vergleichende Gesamtsterin- und Sterinesterbestimmungen der Haut des wachsenden und erwachsenen Organismus, *Ztschr. f. Kinderh.*, **50**:596–607, 1931.
68. NADEL, A. Chemische Studien an der menschlichen Haut. II. Untersuchungen an der normalen und pathologischen Menschenhaut, *Arch. f. Dermat. u. Syph.*, **165**:507–24, 1932.
69. NATHAN, E., and STERN, F. Über den Mineralgehalt der Haut unter normalen und pathologischen Verhältnissen. I. Micromethode zur quantitative Bestimmung des Kalium- und Kalziumgehaltes der Haut, *Dermat. Ztschr.*, **53**:451–56, 1928.
70. ———. Über den Mineralgehalt der Haut unter normalen und pathologischen Verhältnissen. II. Über den Kalium-, Kalzium-, und Wassergehalt der normalen Menschenhaut, *ibid.*, **54**:12–18, 1928.
71. ———. Über den Mineralgehalt der Haut unter normalen und pathologischen Verhältnissen. III. Über den Kalium-, Kalzium-, und Wassergehalt pathologisch veränderter Haut beim Menschen, *ibid.*, pp. 232–37.
72. ———. Über den Mineralgehalt der Haut unter normalen und pathologischen Verhältnissen. IV. Über den Einfluss experimenteller Entzündungen auf den Kalium-, Kalzium-, und Wassergehalt der Kaninchenhaut, *ibid.*, **55**:13–22, 1928.
73. ———. Über den Mineralgehalt der Haut unter normalen und pathologischen Verhältnissen. V. Über den Chlor- und Wassergehalt der normalen und experimentell durch Quarzbestrahlung entzündeten Kaninchenhaut, *ibid.*, **57**:272–77, 1929.
74. ———. Über den Mineralgehalt der Haut unter normalen und pathologischen Verhältnissen. VI. Über Schwankungen in Kalium-, Kalzium-, Chlor-, und Wassergehalt der Kaninchen- und Meerschweinchenhaut bei gleichmässiger Ernährung und bei Hunger und Durst, *ibid.*, **60**:299–313, 1931.
75. PETERS, J. P. Body water: the exchange of fluids in man. Springfield, Ill.: Charles C Thomas, 1935.
76. POPPER, A. Über das Verhalten der Haut schilddrüsenloser Tiere. Inaugural dissertation, Giessen, 1927.
77. ROTHMAN, S. Therapie der Hauttuberkulose. In: BERBERICH and SPIRO, Therapie der Tuberkulose, pp. 655–88. Leiden: A. W. Sijthoff, 1934.
78. ———. Unpublished data.
79. ROTHMAN, S., and SCHAAF, F. Chemie der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1/2**:161–377. Berlin: J. Springer, 1929.
80. ROTHMAN, S., and WEISS, J. Calciumbelastungsproben bei Sklerodermie, *Klin. Wchnschr.*, **10**:1585–86, 1931.
81. ROTHSTEIN, M. Zur Frage der Kochsalzretention, *Klin. Wchnschr.*, **57**:154–56, 1926.
82. SAKATA, S. Über Änderung der Chlor- und Wasserverteilung im tierischen Körper unter Coffeinwirkung, *Arch. f. exper. Path. u. Pharmakol.*, **105**:11–26, 1925.
83. SKELTON, H. P. Water depots of the body, *Proc. Soc. Exper. Biol. & Med.*, **23**:499–500, 1926.
84. ———. The storage of water by various tissues of the body, *Arch. Int. Med.*, **40**:140–52, 1927.
85. STERN, F. Über den Einfluss von Hunger und Flüssigkeitseinschränkungen auf die Kalium-, Kalzium-, Chlor- und Wasserverschiebungen, *Dermat. Ztschr.*, **61**:384–89, 1931.
86. ———. Ernährung und Transmineralization in der Haut, *Arch. f. Dermat. u. Syph.*, **164**:573–602, 1932.
87. STEWART, J. D., and ROURKE, G. M. The effects of large intravenous infusions on body fluid, *J. Clin. Investigation*, **21**:197–205, 1942.
88. SUNTZEFF, V., and CARRUTHERS, C. The mineral composition of human epidermis, *J. Biol. Chem.*, **160**:567–69, 1945.

89. SUNTZEFF, V., and CARRUTHERS, C. The water content in the epidermis of mice undergoing carcinogenesis by methylcholanthrene, *Cancer Research*, **6**:574-77, 1946.
90. SUNTZEFF, V.; CARRUTHERS, C.; and COWDRY, E. V. Influence of age on calcium in epidermal carcinogenesis induced by methylcholanthrene in mice, *Cancer Research*, **5**:572-75, 1945.
91. SZODORAY, L.; VÉRTES, T.; RÁCZ, S.; and HORVÁTH, G. Über einige aktuelle Fragen der Pathogenese des Pemphigus, *Dermatologica*, **102**:125-35, 1951.
92. TALBOTT, J. H.; LEVER, W. F.; and CONSOLAZIO, W. V. Metabolic studies on patients with pemphigus, *J. Invest. Dermat.*, **3**:31-68, 1940.
93. URBACH, E. Zur Pathochemie des Pemphigus, *Arch. f. Dermat. u. Syph.*, **150**:52-70, 1926.
94. ———. Beiträge zu einer physiologischen und pathologischen Chemie der Haut. II. Der Wasser-, Kochsalz-, Reststickstoff-, und Fettgehalt der Haut in der Norm und unter pathologischen Verhältnissen, *Arch. f. Dermat. u. Syph.*, **156**:73-101, 1928.
95. ———. Beiträge zu einer physiologischen und pathologischen Chemie der Haut. I. Ziele und Wege der Gewebschemie. Technik, Literatur, *Zentralbl. f. Haut. u. Geschlechtskr.*, **26**:217-33, 1928.
96. ———. Einfluss der kochsalzarmen Diät auf den Chlorgehalt der Haut, *Compt. rend. d. séances, VIII^e Cong. internat. de dermat. et syph.*, pp. 476-77. Copenhagen: Engelsen & Schrøder, 1931.
97. VAN SCOTT, E. J. Mechanical separation of the epidermis from the corium, *J. Invest. Dermat.*, **18**:377-79, 1952.
98. ———. Personal communication.
99. WAHLGREN, V. Die Bedeutung der Gewebe als Chlordepots, *Arch. f. exper. Path. u. Pharmacol.*, **61**:97-112, 1909.
100. WATERMAN, N. Physikalisch-chemische Untersuchungen über das Karzinom, *Biochem. Ztschr.*, **133**:535-97, 1922.
101. WEISS, H. V.; SILVER, V. E.; and BUECHLER, P. R. A quantitative relationship between the water content of skin and inflammations produced by chemical irritants, *J. Clin. Investigation*, **30**:1507-11, 1951.
102. WYNN, W., and HALDI, J. Further observations on the water and fat content of the skin and body of the albino rat on a high fat diet, *Am. J. Physiol.*, **142**:508-11, 1944.
103. ZHEUTLIN, H. E. C., and FOX, C. L. Sodium and potassium content of human epidermis, *Arch. Dermat. & Syph.*, **61**:397-400, 1950.

CHAPTER 22

Pigmentation

By ALLAN L. LORINCZ

I. SKIN COLOR	516
A. Methods of Measurement	516
B. Determining Factors	516
1. Primary Pigments	516
2. Light-scattering Phenomenon	517
3. Anatomical Variations	518
C. Racial Differences	518
II. SKIN PIGMENTS OTHER THAN MELANIN	518
A. Trichosiderin	518
B. Melanoid	519
C. Carotene	519
D. Hemoglobin Pigments	519
III. MELANIN	519
A. Definition and Functions	519
B. Elementary Composition and General Properties	520
C. Enzymatic Nature of Formation	520
D. Meirowsky Phenomenon	522
E. Tyrosinase Concept for Melanin Formation	523
1. Chemistry of Tyrosinase Reaction	523
2. Role of Redox Potential	527
3. Points of Tyrosinase Action	528
4. Properties of Tyrosinase	528
F. Inhibitors of Melanin Formation	531
1. Direct Enzyme Poisons	531
a) Protein Inactivators	531
b) Copper-binding Agents	531
2. Competitive Inhibitors	533
3. Induction-Period-prolonging Factors	533
4. Compounds Reacting with Melanin Precursors	533
5. Reducing Agents	534
6. Incompletely Understood Inhibitors	534
G. Melanuria	535
IV. BIOLOGY OF DENDRITIC MELANOCYTES	536
A. Anatomical and Embryological Considerations	536
B. Nature of Melanin Granules	538
C. Phenomenon of Pigment Spread	540
V. NUTRITIONAL FACTORS IN MELANIN FORMATION	541
A. Trace Metals	541
B. Amino Acids	542
C. Vitamins	543

VI. ENDOCRINE FACTORS IN MELANIN FORMATION	543
A. Pituitary	544
B. Thyroid	545
C. Adrenal	546
D. Gonadal	547
VII. NEURAL FACTORS IN MELANIN FORMATION	549
VIII. FACTORS INFLUENCING SKIN MELANIN CONTENT	550
IX. SUN-TANNING	552

I. SKIN COLOR

A. METHODS OF MEASUREMENT

IN ORDER to gain knowledge about the factors involved in skin color, numerous attempts have been made to devise objective methods of measuring and recording skin color. These attempts were extensively considered by Jeghers in 1944 in his review of pigmentation of the skin (132). Early methods, now obsolete, relied on visual comparisons and included the use of color scales, such as the universal skin tintometer of Rowntree and Brown (252) and the spinning color wheels and tops widely used in early anthropological studies on racial skin colors (285).

In 1929 the subject of skin-color analysis was placed on a more firm basis when Sheard and Brunsting (262, 33) reported the application of spectrophotometric methods to the analysis of skin color. These methods not only allowed determination of relative luminosity, hue, and saturation of the color factors involved but further enabled actual identification of specific pigments by means of specific absorption changes in spectrophotometric reflection curves. Taussig and Williams (282) used a simplified type of reflectance spectrophotometry involving measurement of light reflected from the skin surface through three successive colored-glass filters.

Edwards and Duntley (62, 63), using an elaborate apparatus—the Hardy recording spectrophotometer—were able to study in quantitative fashion the distribution of pigments in normal skin. They state, briefly, that their apparatus “records the reflectance

of the intact living skin, giving an analysis by wave lengths over the entire visible spectrum, from the violet end at 400 millimicrons to the red end at 700 millimicrons. In the curve thus obtained, each pigment is identified by its characteristic absorption band. The amount of the pigment is indicated by the degree of absorption of the wave length of the band. Lowering of the reflectance at the particular wave length is therefore proportional to the amount of the pigment present.”

B. DETERMINING FACTORS

1. *Primary Pigments*

On the basis of their studies Edwards and Duntley (62) state that the color of normal human skin takes its origin from five primary pigments (Fig. 1). These are melanin, having a general marked absorption at the violet end of the visible spectrum, with no clear-cut absorption bands; oxyhemoglobin, with sharp absorption bands at 542 and 576 $m\mu$; reduced hemoglobin, with a single broad absorption band at 556 $m\mu$; carotene, with a distinct absorption band at 482 $m\mu$; and melanoid, a pigment having some general absorption at the violet end of the spectrum like melanin but, in addition, having a distinct absorption band at 400 $m\mu$. The physical distribution patterns of these pigments and the thickness and optical properties of the overlying colorless tissue layers are additional factors that modify the final color effects produced by these pigments.

Most recently Goldzieher *et al.* (95) have devised a more practical simplified apparatus for skin reflectance spectrophotometry and have confirmed Edwards and Duntley's findings. Furthermore, they extended reflectance studies into the ultraviolet region, where an absorption band at $290\text{ m}\mu$ was identified as being due to soft keratin. Hard

fying skin color. This phenomenon can be defined as the rearrangement of light as it traverses a turbid medium so that the transmitted light contains a larger proportion of long wave lengths (red), whereas the light scattered to the sides and back to the source contains a correspondingly larger proportion of the shorter wave lengths

REFLECTANCE OF NORMAL SKIN AND ITS COMPONENT PIGMENTS

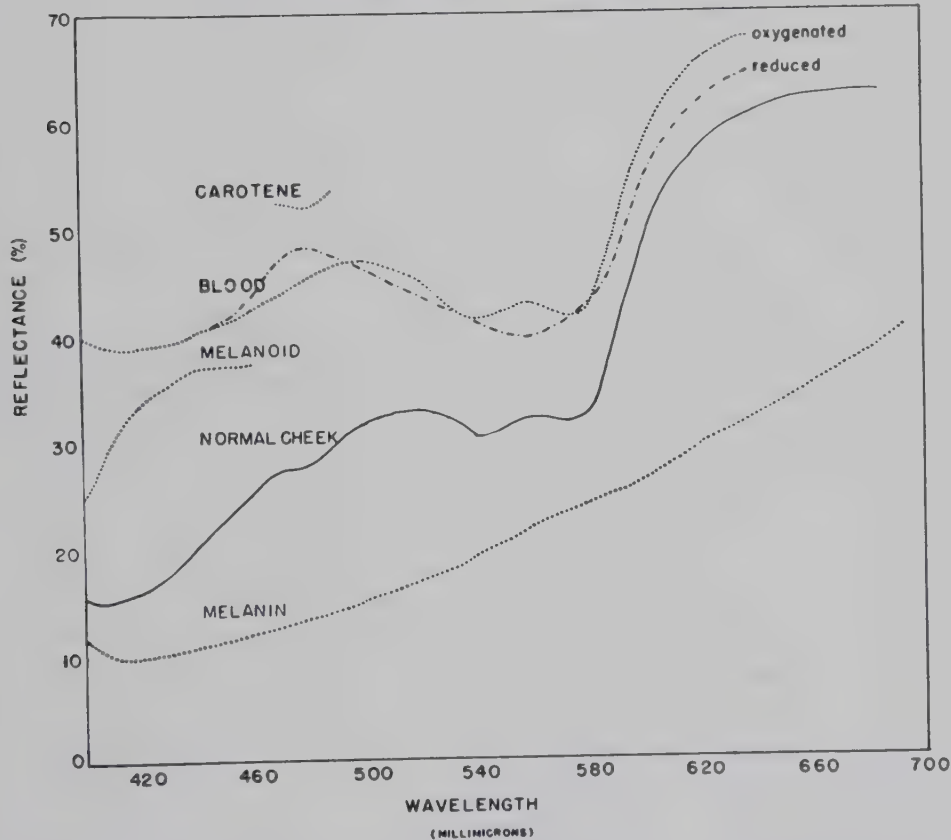


FIG. 1.—Reflectance of normal skin and its component pigments. From Hall, McCracken, and Thorn (111). (Reproduced by permission of Dr. Thorn and the Journal of Clinical Endocrinology.)

keratins and elastin do not show this particular band, which, according to the authors, is probably caused by sulfhydryl-containing amino acids. These workers also noted that melanin absorbs generally, without specific bands, from $470\text{ m}\mu$ down through the ultraviolet range.

2. Light-scattering Phenomenon

Edwards and Duntley (62) emphasized the importance of the phenomenon of light scattering (Tyndall effect) as a factor modi-

(blue). This simply means that blue light is more readily scattered by tiny particles than red light is. The blue color of the sky depends on this scattering phenomenon by tiny atmospheric particles. In the skin the relatively transparent stratum corneum scatters light only slightly, whereas the rete and deeper layers show the effect to a marked degree. Thus the farther below the skin surface any dark pigment is situated, the bluer it appears because of this scattering effect of light in the overlying turbid

tissue layers. The bluish color of blue nevi, Mongolian spots, the black ink of tattoos, and purpuric lesions, for example, are caused by this phenomenon.

3. *Anatomical Variations*

Anatomical variations also obviously modify skin color in other ways. Thus, in general, thin epidermis is more transparent and allows the color of blood pigments in the dermal papillae to shine through more readily than does thick epidermis. Thicker epidermis is less transparent and looks yellower. The degree of development of the granular layer no doubt greatly contributes to the opacity of the epidermis.

Edema of the skin also influences its color, viz., the general pallor associated with the edematous skin of the nephrotic syndrome. The whiteness of macerated skin can serve as another example.

C. RACIAL DIFFERENCES

Clearly evident racial and other differences in skin color are claimed to depend

only on quantitative differences in the various factors involved and chiefly upon variations in the amounts of melanin and melanoid that are present (62). No qualitative pigmentary differences have yet been demonstrated in human skin, no matter what the racial type, although really exhaustive studies on this point are not available. Such qualitative cutaneous pigment differences do occur in mammals other than man. Fetal mouse skin from tan-colored stocks, for example, contains a pigment believed to derive from tryptophane, because skin extracts from such mice will oxidize tryptophane to a tan pigment, whereas similar extracts from other mouse stocks fail to do so (79). Also in guinea pigs there are definite spectral absorption differences between the black or tyrosine-derived skin pigments of certain stocks and the intense yellow-red pigment obtained from other stocks. These two types of guinea-pig pigmentation have, furthermore, been shown to be under the control of entirely independent gene systems (93).

II. SKIN PIGMENTS OTHER THAN MELANIN

A. TRICHOSIDERIN

Besides the five basic cutaneous pigments, an additional unique iron-containing brown pigment—trichosiderin—has been found in normal human red hair (77, 248). Red hair of other mammals does not contain this pigment in easily demonstrable amounts. Nickerson (209), however, reported on the occurrence of a similar pigment in red and buff feathers of several breeds of domestic fowl.¹ Whether trichosiderin also occurs in the characteristic freckles of "redheaded" individuals still remains to be determined. Also the role, if any, that this iron pigment plays in giving red hair its characteristic color is unknown.

1. These observations on trichosiderin in red hair and feathers have more recently been confirmed in every major point by use of a chromatographic method of purification of the pigments (see J. R. Smyth, J. W. Porter, and B. B. Bohren, A study of pigments from red, brown and buff feathers and hair, *Physiol. Zool.*, **24**:205-16, 1951).

It is, of course, not the true red pigment of red hair, because even after extraction of the iron pigment the red color of the hair is preserved. The true red color of human red hair is probably closely related to melanin. Some authors have felt that hair color, whether brown, red, blond, or black, is dependent only on the number, size, and distribution of brown melanin granules in the hair (146, 35), whereas others have considered the various color shades, especially red, to be based upon different oxidation states of melanin (130, 254). Red precursors and red oxidation products of melanin have, of course, been demonstrated in vitro and in melanotic urines.

Recent work by Hanna (116) suggests that trichosiderin may not be a natural pigment at all but only an iron complex of a pigment produced artificially by the interaction of keratin and tryptophane in 6 N hydrochloric acid. Even should this sugges-

tion be true, the high iron content of red hair still remains a unique feature (61).

B. MELANOID

The status of melanoid is rather uncertain. At first, Edwards and Duntley deduced it to be a degradation product of melanin, because it was found normally only in heavily cornified areas, such as plantar and palmar skin, and elsewhere only after a great deal of melanin had been formed, as in areas several months after irradiation by sunlight. Later, however, together with Hamilton and Hubert (63), they noted that testosterone injections in castrate or eunuchoid men caused striking increases in melanoid, with only slight increases in melanin. Melanoid production in large quantities was also stimulated consistently in eunuchs' skin by ultraviolet irradiation, although melanin production was not.

Whether melanoid is the same as the *Hornfarbe* of early authors (135), a well-known dark oxidation product developing in keratin, is not clear, but this seems a very likely possibility. The darkness of the scales in ichthyosis is a classic example of such *Hornfarbe*, as are the dark tips of comedones.

C. CAROTENE

Carotene adds the strong yellow component to normal skin color. It is, of course, the pigment that is closely related to vitamin A and is responsible for the yellow color of butter and carrots. Women in general show much more carotene throughout the skin than do men, and, in addition, women have considerable amounts of this pigment in the breasts, abdomen, and buttocks, which are areas of minimal carotene deposition in the male (62).

Carotene is stored in lipid-rich areas. Thus it occurs not only in dermal and subcutaneous fat but also in the horny layer.

Deposition in the latter is, of course, responsible for the yellow discoloration of the palms and soles in clinical carotenemia.

In untreated castrates Edwards *et al.* (63) found excessive amounts of carotene in the skin. Administration of testosterone brought this pigment back to normal levels, although a temporary increase was noted in the stratum corneum of the palms and soles, which was taken to indicate that some carotene was lost in desquamated cells.

D. HEMOGLOBIN PIGMENTS

Oxyhemoglobin imparts a red component to skin color. This component is especially evident over areas where there is a rich arterial supply and the subpapillary-capillary plexus and the dermal papillae with their capillaries approach closely to the surface. These areas include the flush parts of the face and neck, the nipples, palms, soles, regions of the tuber ischii, and extensor surfaces of the joints. Factors tending to suppress the oxyhemoglobin color component are: (1) venous blood, with its bluish reduced hemoglobin in the venous limbs of the papillary-capillary loops and subpapillary plexuses; (2) melanin, which, by absorbing across the spectrum, blunts the reflection bands of oxyhemoglobin (this factor, of course, gives rise to the difficulty in detecting erythema in dark-skinned Negroes); and (3) the turbidity of the epidermis, which, because of the Tyndall effect, raises the blue end of the reflectance spectrum.

Reduced hemoglobin contributes a dark-bluish or purple component to skin color and was noted in Edwards and Duntley's studies especially in the dorsum of the feet and the lower parts of the trunk. In pathologic cyanosis the characteristic bluish color, of course, comes from reduced hemoglobin. The role of blood circulation in skin coloration is discussed more fully on pages 69 ff.

III. MELANIN

A. DEFINITION AND FUNCTIONS

Of the major skin pigments, melanin will be considered in greatest detail. In reflect-

ance spectrophotometric studies its maximum absorption is in the ultraviolet. Although no more truly precise definition of

the term "melanin" can be given than "dark pigment," its modern use has been more or less limited to the brown or black polymers that originate as oxidation products of tyrosine or orthodihydroxyphenyl compounds. Ropshaw's concept of melanin as the product of the union of protamine and cysteine (239) is mentioned now, only to point out the need for such a limiting definition. His concept will be discussed more fully later. Mason (182) attempted to clarify the concept of melanin by classifying the various types of pigments that have been called "melanin" into two major categories—natural and synthetic—and proposed further subdivisions in these major classes on the basis of their sources and modes of preparation.

Melanin is found throughout the biological world, serving mainly to provide coloration. This pigmentary function has nevertheless been elaborated into important and complex mechanisms for camouflage and sexual attraction. The elaborate nature that the camouflage mechanisms may take can be illustrated by the variety of striped, spotted, and seasonally changing coat patterns of animals, by the "ink" of squids, and particularly by the remarkable adaptive color changes possible in some reptiles, amphibians, and fishes. The flounder, in particular, has very well-developed control over the dispersion of pigment granules in its chromatophores via precise neural and hormonal mechanisms. Some varieties of these bottom-dwelling fish can accurately mimic not only the color shade but also the pattern of their backgrounds. Experimentally, even a checkerboard pattern can be remarkably well duplicated (279). The nature of the neural and hormonal control over chromatophores will be considered more fully later.

The pigmentary nature of melanin further serves to offer some protection against harmful excessive solar radiation and constitutes an important component of eye structure for efficient function. In addition, man has, of course, attached considerable cosmetic and social importance to melanin pigmentation.

In the Negro adult man the total amount of melanin by weight in the body has been estimated to be about 1 gm. (66).

B. ELEMENTARY COMPOSITION AND GENERAL PROPERTIES

Early chemical analyses of melanin showed it to be a high-molecular-weight dyestuff insoluble in water and in most organic solvents.² Elementary analyses (128, 23), though not all in precise agreement, indicated the presence of carbon, nitrogen, hydrogen, and oxygen in approximate proportions of 57, 9, 4, and 30 per cent respectively. Some workers also reported varying smaller amounts of sulfur, iron, and copper; but these can best be considered, as they were by Bloch (25), to be impurities resulting from incomplete removal of attached protein and cellular material. The chemical purification of natural melanin is admittedly a difficult and uncertain task. The ability of melanin to reduce silver and gold salts and osmium tetroxide and the ability of its breakdown products to give positive tests for the indole nucleus were also recognized by early workers, as was the bleaching action of hydrogen peroxide and potassium permanganate on the pigment. In general, however, melanin was found to be rather inert chemically.

C. ENZYMATIC NATURE OF FORMATION

Serious study of the chemistry of melanin began after Bloch's discovery of the famous dopa reaction (22, 23, 27). He immersed frozen sections of pigmented human skin into a dilute solution of *l*-3,4-dihydroxyphenylalanine ("dopa") at pH 7.3–7.4 and noted that brown granules appeared in the cytoplasm of scattered cells in the basal layer (Fig. 2). He further closely correlated

2. Solubility in alcohol and pyridine was reported by Bloch and Schaaf (27), and solubility in ethylene diamine and ethylene chlorohydrin was noted by Lea (152). In these reports, however, it is probable either that denatured melanins were involved or that the solvents reacted chemically with the pigment polymer to break it down. Solubility in acids of alkali-precipitable melanin from melanuric urine is also well known.

the intensity of this dopa reaction with known varying melanin-forming capacities of skin from different sources. The enzymatic nature of the dopa reaction was then deduced from the following features: It was abolished by boiling the skin or in the presence of enzyme poisons, such as hydrogen sulfide, sulfur dioxide, and fairly high concentrations of potassium cyanide; it was highly specific, requiring the levorotatory form of dopa; and it had a definite pH range, being abolished in acids and having an

claimed shortcomings of the theory (192): Cells other than melanin-forming cells were dopa-positive, including leukocytes, nerve fibrils, striated muscles, ganglion cells, sweat glands, mast cells of mice, red blood cells, and keratohyalin; and even fruits, such as apples, and bacteria and fungi could be stained with dopa. The dopa reaction was nonspecific, because dopa-positive cells could also be stained by rongalite-white, paraphenylene diamine, and several other easily oxidizable substances which were

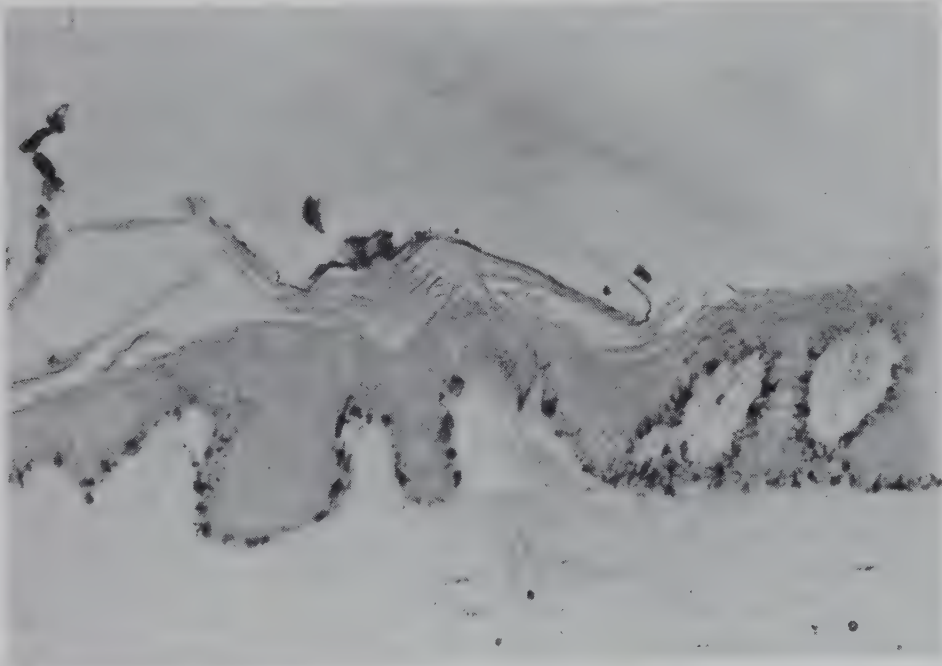


FIG. 2.—Dopa stain, showing dopa-positive cells in basal layer

optimum pH of 7.35. On the basis of this work Bloch considered melanin to be a polymerized enzymatic oxidation product of dopa and named the hypothetical pigmentogenic enzyme in the skin “dopa-oxidase.”³

This dopa-oxidase concept, however, was vigorously challenged by a group led by Meirowsky. They pointed out the following

3. Recently Van Duijn has challenged some of Bloch's findings about the lability of the histochemical dopa reaction in skin, which have, according to him, led to its premature classification into the group of copper-containing phenolase catalyzed reactions (P. Van Duijn, Inactivation experiments on the dopa factor, *J. Histochem. & Cytochem.*, 1:143-50, 1953).

clearly nonmelanin-forming agents. Furthermore, Thannhauser and Moncorps (196), contrary to Bloch's findings, claimed that freshly pigmented skin reacted with several catechol derivatives other than dopa, including *p*-hydroxyphenylpyruvic acid, homoprotocatechuic acid, and 3,4-dihydroxyphenylpyruvic acid. Skin extracts were said to react not only with dopa but also with adrenaline, epirenin, catechol, hydroquinone, *d*-3,4-dihydroxyphenylalanine, and sometimes even *p*-cresol and tyrosine (255). Specific dopa-oxidase activity could not be demonstrated in skin extracts by usual manometric enzymatic technics. Meirowsky

(191, 192), hence, denied that there was a specific dopa-oxidase in skin and claimed that Bloch's results could be explained by nonspecific polyphenolase activity. All facets of this dispute cannot be considered here, but it should be pointed out that this controversy served as a potent stimulus for research and that much valuable basic information about melanogenesis was uncovered by both sides.

D. MEIROWSKY PHENOMENON

Meirowsky (190) discovered a phenomenon which bears his name and which is characterized by an increase in epidermal pigment in excised or cadaver skin when it is incubated at 37° C. and higher or on exposure to ultraviolet light. This process was further studied by Sharlit (260), who noted that atmospheric oxygen was unnecessary for the phenomenon to occur and that boiling the skin or formalin fixation did not alter it. Furthermore, cyanide and fluoride ions enhanced the process, whereas mercuric ions inhibited it. Sharlit also noted that the pigment produced as a result of the Meirowsky phenomenon never appeared in the form of dendritic cells but was seen as a granular deposit chiefly around the periphery of cells in the lower layers of the epidermis. The exact role that the Meirowsky phenomenon plays in human skin pigmentation is difficult to assess. At least the so-called "pigment darkening" or immediate pigmentation developing within an hour after ultraviolet light exposure is probably a result of this process. This immediate darkening is more pronounced in areas already slightly pigmented diffusely from ultraviolet light (62) or in freckles (68).

Various attempts to harmonize the Meirowsky phenomenon with the Bloch dopa-oxidase concept have been made. Thus Miescher and Minder (194) claimed that it resulted from oxidation and consequent darkening of pale, incompletely oxidized or reduced, preformed melanin granules. Sharlit (260) considered that it was caused by a shift of cellularly active oxygen from "priority" respiratory enzyme systems, which

were destroyed by factors which elicit the phenomenon, to the melanin-forming system. Others went so far as to consider the phenomenon an artifact, the result of tissue shrinkage (261), with consequent closer clustering of melanin granules already present, or to state that it was merely analogous to the kind of nonspecific tissue darkening seen in gangrene.

Ropshaw (239), on the other hand, elaborated an entirely different theory for melanogenesis which could encompass the Meirowsky phenomenon. He claimed that melanin formation came about as the result of nuclear cleavage followed by union of protamine liberated from the nucleus, with cysteine present in the cytoplasm to form a black precipitate if the cellular pH was sufficiently alkaline. He had mixed protamine extracted from skin with reduced glutathione at pH 7.38 and found that a black precipitate appeared. However, he found various unidentified contaminating microorganisms in those of his preparations in which the reaction occurred. He further found that iron catalyzed this reaction between protamine and cysteine and that it was nonenzymatic, because boiling of the reagents did not alter the results. Although Ropshaw's experiments were compatible not only with the nonenzymatic Meirowsky phenomenon but also with Meirowsky's contention that melanin formation commenced from cellular nuclei—a view he still holds (193)—his postulate did not receive recognition or support (except by Latarjet [150]), chiefly because of the complicating factor of contaminant microorganisms and because no chemical analyses of the resulting black precipitates were made. Certainly, very little can be said about the relation, if any, of Ropshaw's incomplete, nonphysiologic, test-tube experiments to melanogenesis in the living skin. This work of Ropshaw is mentioned chiefly to point out the difficulties engendered by generalizing the definition of melanin to include any black pigment occurring in nature or in the test tube as the result of a variety of manipulations.

E. TYROSINASE CONCEPT FOR MELANIN FORMATION

Considerably antedating the dopa-oxidase controversy, Bourquelot and Bertrand (31), near the turn of the century, discovered an enzyme in the mushroom *Russula nigricans* which oxidized tyrosine in that plant through red intermediate stages to a brown-black pigment having many properties of melanin. They named this enzyme "tyrosinase" (16). Following discovery of this enzyme, it was found widely distributed in nature, in numerous plants, bacteria, invertebrate animals, and fish. It was held responsible, in many instances, for the pigmentation of these organisms. As early as 1901 von Fürth (87) had suggested that such an oxidative enzyme might be responsible for melanin formation. Kastle in his review lists numerous sources of tyrosinase (139).

For a long time sporadic and contradictory reports appeared about the presence of tyrosinase in melanin-forming mammalian tissues, especially in melanotic tumors. Positive results were reported by some (90, 59, 305, 4, 227, 52) and negative findings by others (23, 215, 310, 40, 253). This confusion about mammalian tyrosinase began to clear in 1942, when Hogeboom and Adams (124) found transplantable mouse melanomas to contain tyrosinase. Subsequently, this finding received conclusive confirmation from many sources. The properties of mammalian tyrosinase were studied and reported in detail by Lerner, Fitzpatrick, Calkins, and Summerson (160). Recently, tyrosinase activity has even been demonstrated histochemically in normal human skin by Fitzpatrick, Becker, Lerner, and Montgomery (74). They irradiated human skin *in situ* for 7 consecutive days with erythema doses of ultraviolet radiation and then excised a biopsy specimen and incubated it in a buffered tyrosine solution and found darkening of melanocytes similar to that occurring in the dopa reaction (Fig. 3).

1. Chemistry of Tyrosinase Reaction

Major sources of tyrosinase for experimental studies have been mushrooms, po-

tatoes, meal-worm larvae, and mouse melanomas. The series of reactions and intermediate products resulting from tyrosinase action were uncovered largely through the classic work of Raper and his co-workers (232). They allowed meal-worm tyrosinase to act upon tyrosine at pH 6.0 until the maximal red intermediate-color stage was reached. The enzyme was then precipitated with acetic acid and removed. On decolorization of the remaining red solution, either by allowing it to stand in vacuo or by adding sulfurous acid, they could demonstrate the presence of three substances in the solution—*l*-3,4-dihydroxyphenylalanine, 5,6-dihydroxyindole, and 5,6-dihydroxyindole-2-carboxylic acid. The indole compounds, being unstable, were identified by methylation under hydrogen with dimethyl sulfate to the corresponding dimethyl compounds. The decarboxylated indole predominated when the red substance had been allowed to decolorize spontaneously, whereas the carboxylated indole compound predominated when the decolorization was speeded up with sulfurous acid. When *l*-3,4-dopa was used as the substrate for the enzyme, the same indole derivatives were obtained. The conclusion was therefore reached that dopa represented the first step in the enzymatic oxidation of tyrosine. Largely on the basis of these findings, Raper and his group then proposed the well-known series of reactions for the events occurring in the tyrosinase-tyrosine reaction (Fig. 4).

Dopaquinone (228) has never been directly identified in the reaction. It is very unstable and, if actually formed, is believed to undergo immediate intramolecular rearrangement in a fashion analogous to anilinoquinone formation, so that the nitrogen of the side chain links up with the 6 position of the benzene nucleus to form 5,6-dihydroxy-dihydroindole-2-carboxylic acid (reduced dopachrome or "leucobody") (231). That orthoquinone formation can be brought about by tyrosinase action has been demonstrated by allowing the enzyme to act upon catechol in the presence of aniline. Under these circumstances the anilinoquinone de-

rived from orthobenzoquinone can be isolated. To explain further the difficulty in obtaining dopaquinone from the tyrosinase-tyrosine reaction, Evans and Raper (67) have pointed out that the oxidation-reduction potentials are such that reduced dopachrome

dopaquinone after noting that dopa accumulates initially in the tyrosinase-tyrosine system. After 2–5 hours they found that 10–20 per cent of the tyrosine oxidized by meal-worm tyrosinase could be recovered as dopa, even though the enzyme can oxidize

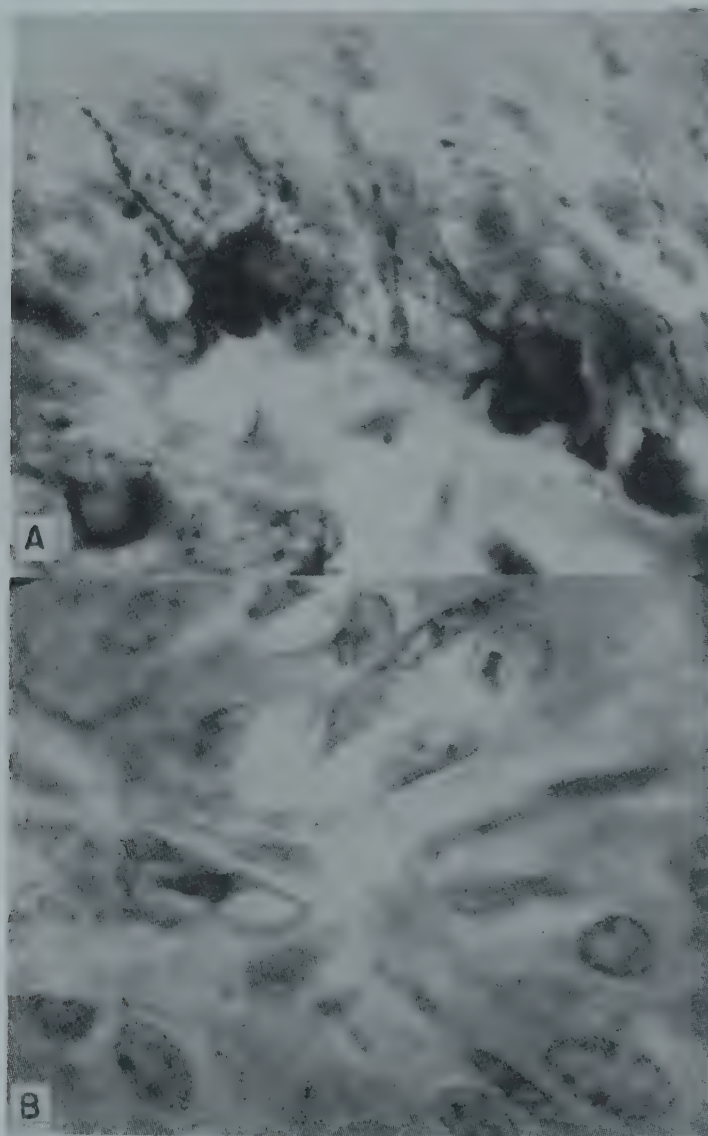


FIG. 3. Activation of tyrosinase by ultraviolet light. *B*, normal white human skin incubated with tyrosine. No melanin formation is observed. *A*, skin from the same individual after exposure to ultraviolet light in vivo and then incubation in tyrosine in vitro. Melanin is formed at the site of tyrosinase and outlines the melanocytes. From A. B. Lerner and T. B. Fitzpatrick, *The control of melanogenesis in human pigment cells*. In: *Pigment cell growth* (New York: Academic Press, Inc., 1953), pp. 319–33. (Reproduced by permission of Dr. Lerner and Academic Press, Inc.)

immediately reduces any dopaquinone present back to dopa and is itself oxidized in the process to red dopachrome, the first visible product of the enzyme's action. They postulated this action of reduced dopachrome on

dopa more easily than tyrosine; and they also noted that the addition of reduced dopachrome increased this accumulation of dopa.

For a long time dopachrome—the qui-

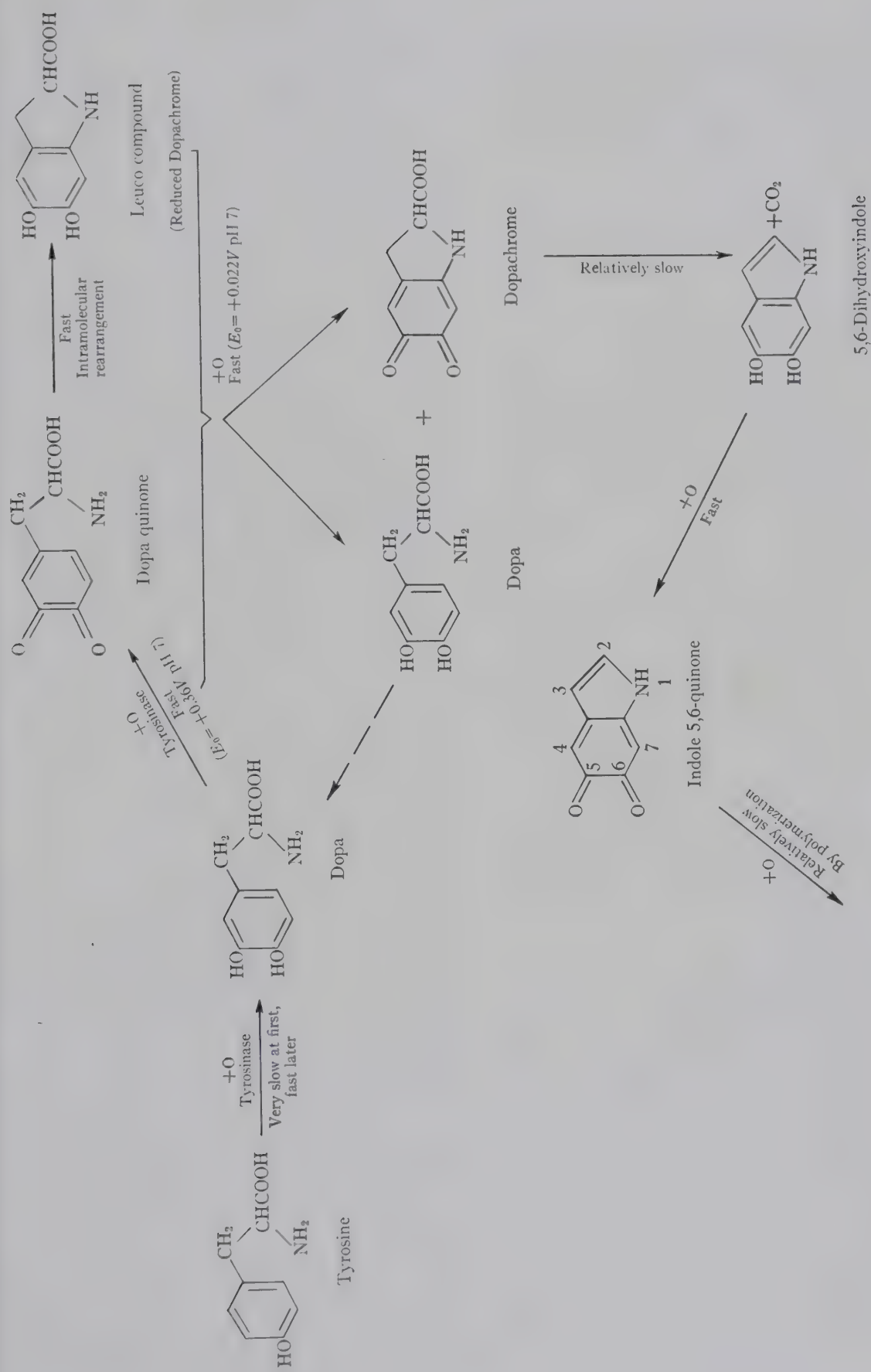


FIG. 4.—Raper's scheme of melanin formation, modified

none of 5,6-dihydroxydihydroindole-2-carboxylic acid—was believed to be identical with hallachrome, which occurs naturally in the worm *Halla parthenopaea* (189). Bu'lock, Harley-Mason, and Mason (34) have, however, recently brought forth spectrophotometric evidence to prove that these substances are dissimilar, and hence they proposed the name "dopachrome" for the red intermediate compound in the melanin-forming reaction.

The last step in the Raper scheme for melanin formation has two alternative possibilities, one involving decarboxylation prior to polymerization and the other not. The decarboxylative mechanism is more likely under normal conditions for the following reasons: (1) sulfurous acid was shown by Raper to be necessary for nondecarboxylative melanin formation to be favored in tyrosinase-tyrosine systems; (2) rabbits injected with dihydroxyindole excrete a conjugated chromogen in the urine identical with that found in melanuric urine, whereas when 5,6-dihydroxyindole-2-carboxylic acid is similarly injected, it is excreted unchanged; and (3) Mason (187) has shown manometrically that decarboxylation and rearrangement of dopachrome occur simultaneously and that this is not an enzymatic process but is dependent on hydroxide ion catalysis—in acid solutions the nondecarboxylative rearrangement is favored.

The steps subsequent to 5,6-dihydroxyindole formation have been recently investigated and discussed by Mason (183). He has shown spectrophotometrically that, in the presence of tyrosinase, 5,6-dihydroxyindole is oxidized to a purple pigment, which is indole-5,6-quinone or its quinonimine tautomer. It is probably this compound whose absorption spectrum was given by Mazza and Stolfi (189) when they described "hallachrome."

Several methods of polymerization of melanin precursors have been suggested in the past. Mason (183) reviewed these early proposals and found them nonapplicable to enzymatic melanin formation. He rather regards indole-5,6-quinone as at least a bi-

functional monomer capable of undergoing coupling with itself.

Several groups of workers, especially in Britain, have recently undertaken a detailed investigation of this problem of melanin polymerization by synthesizing many melanin precursors with substituted groups blocking various of the possible reactive positions (184). The problem, however, was found to be more complex than was apparent at first, because several different modes of oxidative polymerization of the substituted hydroxyindoles can occur, depending on which positions are blocked. Thus if the one position is blocked with a methyl group by starting with N-methyl tyrosine, an N-methyl melanin can be formed. Clemo and Duxbury (43), however, have shown that the ultraviolet absorption spectrum of the melanin derived from this N-methyl tyrosine differs radically from that of tyrosine melanin. Incidentally, the melanin-like substance derived from adrenaline via oxidation through adrenochrome by tyrosinase is such an N-methyl polymer, and thus Wohlge-muth's (306) claim that adrenaline is the precursor of natural melanin cannot be accepted unless natural melanin is shown to have an N-methyl tyrosine type of absorption spectrum, which has not yet been done.

About the 2 position we know that the polymerization of unsubstituted 5,6-dihydroxyindole on alkaline oxidation yields a melanin which gives a negative Ehrlich *p*-dimethyl-amino-benzaldehyde reaction, whereas if 5,6-dihydroxy-2-methylindole polymerizes under similar conditions, it gives a black precipitate (37) with a positive Ehrlich test (38). The spectrum of 2-methyl-substituted 5,6-dihydroxyindole polymer also differs markedly from that of ordinary tyrosine melanin. If both the 1 and 2 positions of the potential indole nucleus are blocked, as when the ethyl ester of N-methyl tyrosine is the starting compound, no dark pigment at all is formed by tyrosinase. In view of these experiments, position 2 cannot altogether be excluded from involvement in the polymerization of tyrosine-derived melanin.

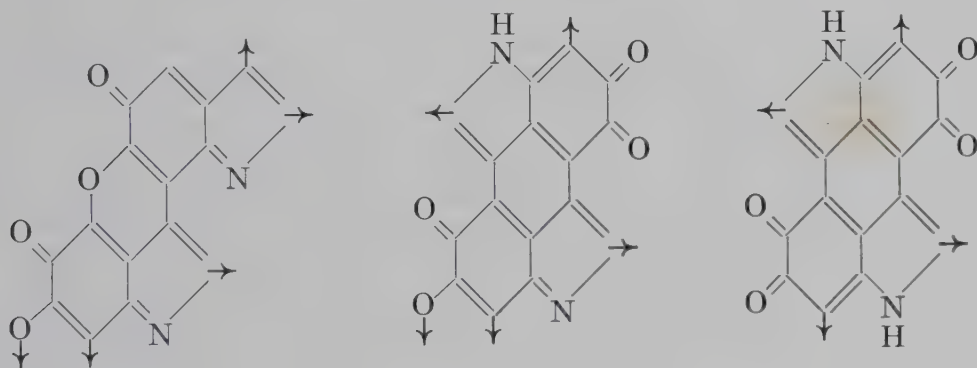
The status in polymerization of position 3 of the indole nucleus is similarly uncertain, because, although 5,6-dihydroxy-3-methylindole which has been prepared and does polymerize on alkaline oxidation, it does so to a blue rather than to a brown or black precipitate (11). Also 5,6-dihydroxy-2,3-dimethylindole has been prepared and darkens on alkaline oxidation but fails to polymerize sufficiently to form a precipitate.

Position 4 need not be involved in polymerization because 2,3-dihydroxyphenylalanine can still lead to the formation of a black precipitate with a tyrosine-melanin type of absorption spectrum (43). This observation indicates that the 2 hydroxy group of the 2,3-dihydroxyphenylalanine, which becomes the 4 hydroxy on the indole nucleus, can remain free during the process.

The role of the 5 and 6 positions has been

clarified only to the extent that orthoquinone formation at these positions is not essential for polymerization, because Morton and Slaunwhite (201) have shown that 6-hydroxy-5-methyl-3-phenylindole-2-carboxylic acid can still polymerize to a melanin-like material. The role of the 7 position was explored by the synthesis of 7-isopropyl-5,6-dihydroxyindole and was found not essential for pigment polymer formation.

Although no definite chemical picture can yet be drawn to illustrate the nature of the melanin polymer, much progress toward such a goal has been made, and active investigation of the subject continues. Mason and Lada (186) most recently have proposed the following structures to illustrate the types of in vitro dopa melanin polymerization units that are possible on the basis of currently known facts.



It is likely that a quinonoid structure exists in the polymer because of the dark color of melanin, which can be reversibly reduced to a light-tan color. Such a reduced light-tan melanin can be obtained by treating black melanin with reducing agents, such as ascorbic acid or sodium hydrosulfite. These tan melanins can be reconverted to the black state by adding an oxidizing agent, such as potassium ferricyanide. This reversible oxidation was earlier demonstrated in histologic sections by Miescher and Minder (194). Extreme oxidation, however, will again bleach melanin, as is clearly illustrated by hydrogen peroxide hair bleaches and by the bleaching action of permanganate and bichromatic ions on melanin.

2. Role of Redox Potential

Figure (70) has considered extensively the oxidation-reduction potentials in the melanin-forming reactions. He noted that tyrosinase functions best at a redox potential of +0.065 volts and that it becomes almost completely inhibited at potentials either above +0.3 or below -0.3 volts, such as can be achieved in the presence of potassium ferricyanide and glutathione, respectively. He further pointed out that the redox potential during in vitro melanin formation is largely poised by the dopachrome system at about +0.022 volts at pH 7, because both oxidized and reduced forms of dopachrome can coexist for appreciable times, owing to the relatively slow molecular rearrangement of

dopachrome to the subsequent melanin precursor. The dopa to dopaquinone redox potential at pH 7 was shown by Ball (8) to be +0.360 volts. This potential, if maintained, would be high enough to inhibit the enzyme, but, as pointed out already, the formation of dopaquinone is merely a transitory, if not a hypothetical, step, and this compound cannot accumulate to any extent because of the reducing action of reduced dopachrome. Because biological oxidations generally proceed in the greatest possible stepwise fashion, it is also probable that transitory intermediate steps, not yet mentioned, involving semiquinone formation occur during the process of melanin synthesis.

From the preceding considerations it is evident that the redox potential at the site of melanin formation must exert a profound regulatory effect on the process.

3. Points of Tyrosinase Action

The enzyme tyrosinase acts at several points in the reaction. Its presence under physiological conditions is essential for the first step involving oxidation of tyrosine to dopa to occur. Arnow (7), however, has shown that even this step can proceed in the absence of enzyme through the action of intense ultraviolet irradiation; and Rothman (243) later showed that much less intense ultraviolet irradiation can accomplish the same thing if ferrous salts are present. Ascorbic acid (242, 290) was also found to catalyze this first step. The first point of tyrosinase action involving oxidation of tyrosine to dopa is marked by an unusual feature known as the "induction period." Thus, when tyrosine and tyrosinase are mixed, a time lag occurs before the maximal rate of oxidation gets under way. This lag is the induction period and has been defined for the tyrosinase-tyrosine system as the intercept on the time axis of an extension of the slope of the oxidation curve when oxidation is proceeding maximally (160). An induction period of this sort is not observed when tyrosinase acts upon dopa as substrate (Fig. 5). Several factors can regulate the duration of the induction period in tyro-

sinase-tyrosine oxidation. Among these the redox potential is an important one. Anything tending to lower it shortens the induction period, whereas agents that raise this potential prolong the induction period. Besides this redox potential factor, the presence of small amounts of *l*-dopa in some way more specifically shortens the induction period. This action was pointed out by Lerner and Fitzpatrick (157), who noted that *l*-dopa was more effective in shortening the induction period than an equal amount of *dl*-dopa. Because of this accelerating action of dopa on the first step of melanin formation, it is evident that this step is an autocatalytic one.

The steps to melanin beyond dopa will proceed slowly via atmospheric oxidation in nonacid solutions, even in the absence of tyrosinase. The presence of tyrosinase, however, greatly hastens these steps. In these phases of the melanin-forming process beyond the initial step, tyrosinase is believed to act at points where orthodihydroxy structures are oxidized to their corresponding orthoquinones, such as dopa to dopaquinone and 5,6-dihydroxyindole to indole-5,6-quinone.

4. Properties of Tyrosinase

The nature and properties of the enzyme tyrosinase itself have been reviewed in considerable detail more recently by Nelson and Dawson (206) and by Lerner *et al.* (160). There is general agreement that certain properties are common to all tyrosinase preparations, regardless of their source or mode of preparation, while other properties show considerable variation. Thus all tyrosinases bring about the oxidation of tyrosine to melanin; all are copper-protein complexes, in which copper is related to enzymatic activity; and, for all, the presence of an orthodihydroxyphenyl compound, such as dopa or catechol, enhances the oxidation of monophenolic substrates, such as tyrosine. As regards this last point, Nelson and Dawson suggest that the monophenolase activity of tyrosinase is secondary to its orthodihydroxyphenol-oxidizing property in such

a way that during the oxidation of an orthodihydric phenol a portion of the enzyme complex becomes activated, so that it can bring about the oxidation of monohydric phenols. Their reasons for proposing this concept of one enzyme with two activities, one of which is activated during the opera-

alone do not bring about monophenol oxidation and chemical removal of such oxidation products does not halt enzymatic monophenol oxidation; and (3) orthodihydroxyphenolic compounds markedly catalyze monophenolase activity. Lerner, Fitzpatrick, Calkins, and Summerson (159, 160,

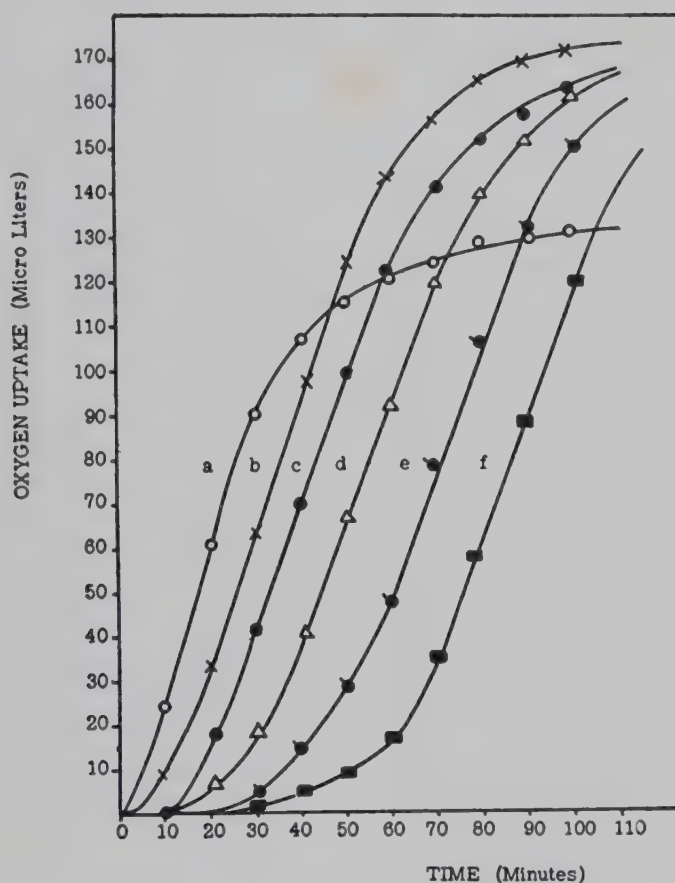


FIG. 5.—The effect of dopa on the induction period in the enzymatic oxidation of tyrosine by mouse melanoma preparations at pH 6.8 and 38°. Curve *a*, 0.5 mg. of dopa; curve *b*, 0.1 mg. of dopa + 0.4 mg. of tyrosine; curve *c*, 0.05 mg. of dopa + 0.45 mg. of tyrosine; curve *d*, 0.01 mg. of dopa + 0.49 mg. of tyrosine; curve *e*, 0.001 mg. of dopa + 0.50 mg. of tyrosine; curve *f*, 0.50 mg. of tyrosine. From Lerner *et al.* (160). (Reproduced by permission of Dr. Lerner and the Journal of Biological Chemistry.)

tion of the other, are based on the following considerations: (1) although great variations in relative monophenolase and dihydroxyphenolase activities can occur in tyrosinases prepared by different methods, the two activities have never been entirely dissociated in even the purest preparations; (2) active participation of enzyme is necessary not only for dihydroxyphenolase but also for monophenolase activity, because oxidation products of dihydroxyphenols

161, 162, 163), working with melanoma tyrosinase, confirmed these findings and brought forth a further observation supporting this one enzyme with two activities concept. They found that N-acetyl and N-formyl-tyrosine, competitive inhibitors of tyrosine-tyrosinase systems, also inhibit dopa-tyrosinase systems.

Among the variable properties of different tyrosinase preparations can be listed the relative potencies of monophenolase and

orthodihydroxyphenolase activities—stability, substrate range, requirement of activators, susceptibility to inactivation during the course of enzyme action, and electrophoretic and other physicochemical characteristics. Among plant tyrosinases, that from mushrooms has been so highly purified by Mallette and Dawson (178) as to be electrophoretically homogeneous and almost so in the ultracentrifuge. This preparation was a copper-protein complex with a molecular weight of approximately 100,000 and contained four atoms of copper per molecule.

Tyrosinase of mammalian origin differs most significantly from tyrosinases of plant and lower animal origin, in that it has not thus far been separated from cellular particulate matter and brought into colloidal solution. Furthermore, it has a more limited substrate range, it is not readily inactivated during the reactions it brings about, and a quantitative relationship has been shown between the negative logarithm of the dopa concentration and the induction period.

Kertesz (142) most recently entirely rejects tyrosinase as a specific enzyme and has brought forth evidence showing that plant tyrosinase, at least, is simply polyphenol-oxidase plus orthoquinone plus metallic ions. Although copper is specifically required for polyphenolase activity, for tyrosinase activity other metallic ions, such as cobalt, nickel, and vanadium, will serve, although copper is about two and a half times as active as these. Iron, zinc, chromium, manganese, and magnesium were found inactive, and, curiously, cytochrome c was found to inhibit the tyrosinase activity of this system.

Mammalian tyrosinase extracted from Harding-Passey mouse melanomas has been studied extensively by Lerner, Fitzpatrick, Calkins, and Summerson (159, 160, 161, 162, 163). Only tyrosine and its ethyl ester, among a number of monohydroxy compounds tested, could serve as suitable substrates. Phenol, di-iodotyrosine, 4-methoxyphenylalanine, and tyramine were relatively inert even in the presence of catalytic amounts of dopa; 3-amino, 3-fluoro-, N-formyl-, and N-acetyl derivatives not only

failed as substrates but proved to be potent inhibitors. The N-acetyl-tyrosine was even proved to be a true competitive inhibitor of tyrosinase activity. Among dihydroxy compounds, only *l*-dopa and, to a lesser extent, *dl*-dopa and *dl*-paredrine were significantly active substrates. Arterenol, catechol, and homogentisic acid could, however, shorten the induction period of enzyme action. Furthermore, these latter compounds and epinephrine, isuprel, hydroquinone, *p*-benzylhydroquinone, and cobefrin could be rapidly oxidized in the system studied if dopa oxidation proceeded simultaneously.

That copper was essential for mammalian tyrosinase activity (161) was shown by adding KCN to the enzyme and dialyzing the copper cyanide complex away, as had been done previously by Kubowitz (147, 148) with potato tyrosinase. This procedure reduced enzyme activity by 85 per cent. This loss of activity could be restored by adding cupric ions; Fe^{+++} , Zn^{++} , Co^{++} , Mg^{++} , and Mn^{++} were ineffective in restoring activity, but Ni^{++} could restore activity to some extent. Other copper reagents, such as sodium diethyldithiocarbamate and phenylthiourea were also found to inhibit this enzyme (159), and their inhibiting action could be released by adding excess copper.

Although Sizer (265, 266) has reported mushroom tyrosinase capable of bringing about oxidation of tyrosine combined in peptide chains in protein, Lerner, Fitzpatrick, Calkins, and Summerson (162) do not believe this likely with mammalian tyrosinase. They point out the inability of this latter enzyme to oxidize the N-acetyl or N-formyl substituted tyrosines in which the amino group is bound in peptide bond fashion. Kausche and Hahn (140) also have been unable to demonstrate tyrosinase oxidation of tyrosine bound in peptide linkages.

The effects of various ions on melanin formation by mammalian tyrosinase were studied by Lerner (156). The monovalent cations—lithium, sodium, potassium, and ammonium—had no effect on *in vitro* melanin formation. Of the anions, the previous finding that chloride in high concentrations

(about three times physiologic) inhibits the process was confirmed, and, furthermore, fluoride, acetate, and oxalate ions were found similarly to inhibit *in vitro* tyrosinase action. Strongly oxidizing anions, like nitrite and persulfate, also interfered with enzyme action, as might be expected from their actions on the redox potential. Sulfate, nitrate, bromide, iodide, and a number of other anions showed no effect. Curiously, however, lithium sulfate was found to inhibit the reaction.

Twenty-four metallic ions were also tested for their ability to interfere with copper reactivation of apotyrrosinase (tyrosinase with its copper removed). Of these, only mercury, silver, and gold ions could compete with copper for position on the apo-enzyme so as to produce an inactive preparation. It was also found that the first metal bound by the apo-enzyme was not easily replaced by the other ions. These experiments suggested a mechanism to explain the well-known mild skin-bleaching action of ammoniated mercury ointments (205). Mercuric ions thus might partially displace copper from tyrosinase and interfere with enzyme action.

The role of silver added to the melanin-forming reactions may be still further complicated. Experiments in my laboratory (12) showed that silver almost completely inhibits oxygen uptake in a dopa-tyrosinase system, whereas, the *rate* of oxygen uptake in a tyrosine-tyrosinase system is not affected, although the total oxygen uptake is diminished by approximately 30 per cent. This would suggest that silver interferes with oxygen uptake in melanin formation at some point beyond the first step of tyrosine to dopa oxidation. This is, of course, likely, in view of the reactivity of silver with orthodihydroxy compounds.

F. INHIBITORS OF MELANIN FORMATION

The enzymatic process of melanin formation can be inhibited by a number of inhibitory factors acting through several mechanisms, some of which have already been briefly mentioned. These inhibitory

factors can be classified into the following categories: (1) direct enzyme inhibitors or poisons, (2) competitive inhibitors, (3) induction-period-prolonging agents, (4) compounds reacting with intermediates in the melanin-forming chain of reactions, (5) reducing agents, and (6) inhibitors of largely unknown modes of action. Some of these inhibitors have been shown to be active *in vivo* as well as *in vitro*.

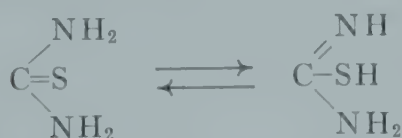
1. *Direct Enzyme Poisons*

The direct enzyme inhibitors or poisons act either by inactivating the protein component of the enzyme or by tying up its essential copper component.

a) Protein inactivators.—Among factors affecting the protein moiety is a large number of nonspecific protein denaturants, such as excessive heat, strong acids and bases, and other powerful chemical reagents. Specific inactivation of the protein component of tyrosinase, however, is also possible by specific tyrosinase antibodies, such as can be produced, for example, against mouse melanoma tyrosinase by injecting it into rabbits (296). Possibly this latter mechanism of pigment inhibition can account for leukodermas associated with sympathetic ophthalmia and Vogt-Koyanagi syndrome (138), and perhaps speculations can even be made along this line in respect to vitiligo.

b) Copper-binding agents.—Inhibitors that inactivate the copper component of the enzyme have been much more extensively studied and are physiologically of much greater importance. Such copper-binding agents act by forming weakly dissociable complexes with this metal. Among these substances are carbon monoxide, cyanide ion, hydrogen sulfide (23), and many organic sulfur compounds, most of which have reactive sulfhydryl groups. Among these organic sulfur compounds are diethyldithiocarbamate, cysteine, glutathione, 2,3-dithio-propanol (BAL), and thiourea and its derivatives, such as phenylthiourea, allylthiourea, α -naphthylthiourea, and thiouracil (157, 124, 14, 58, 218). Although the thioureas do not contain any sulfhydryl

groups, an isomeric isothiurea which has an active sulfhydryl group is formed in aqueous solutions as follows:



The thioureas are active inhibitors of enzymatic melanin formation *in vivo* at times as well as *in vitro*. Among lower vertebrates thiouracil inhibits pigment formation in the regenerating tail of the axolotl (131).

In tissue cultures of chick melanocytes, pigment granule formation was inhibited by phenylthiourea, α -naphthylthiourea, thiouracil, and diphenylthiourea. These inhibitory effects were reversed by adding free iodine or dopa to the cultures (179).

Among mammals, black rats fed α -naphthylthiourea or phenylcarbamide show graying of hair only as long as such substances remain in the diet (234, 54). In man, too, these compounds have inhibited pigment formation. Thus thiouracil given to a patient with metastatic malignant melanoma stopped the associated melanuria (299). Also a Negro patient treated with thiouracil for hyperthyroidism was reported to develop areas of depigmentation (121). Of course, these are just isolated reports, and depigmentation in man is not a general phenomenon after administration of thiouracil or its derivatives. In mice, too, the administration of several thiourea derivatives in massive doses failed to inhibit melanin formation in melanomas (276).

The knowledge that the natural regulation of melanin formation may depend upon organic sulfhydryl compound inhibition of tyrosinase came about in the following manner. First, it was shown that crude aqueous extracts of rabbit or guinea-pig skin could inhibit the oxidation of tyrosine by tyrosinase (255, 256, 93). Rothman and co-workers (249) next were able to demonstrate this inhibitory factor in human epidermis as well and further showed that its inhibitory action could be released by specific sulfhydryl poisons. They therefore concluded that the

inhibition was a function of sulfhydryl compounds in the epidermis and proposed the following hypothesis to account for the natural regulation of melanin formation. In melanocytes both substrate and active enzyme are present but are unable to interact fully because of the inhibitory action of the sulfhydryl groups also present. Pigmentogenic stimuli, such as sunshine, X-rays, heat, and inflammatory skin diseases, act by oxidizing or otherwise destroying the inhibitory sulfhydryl groups, thus permitting the enzyme to act freely on the substrate. Furthermore, nonpigmented skin capable of forming melanin remains nonpigmented because sulfhydryl compounds hinder tyrosinase from acting fully on the substrate.

After this fruitful hypothesis was proposed in 1946, several lines of supporting evidence were brought forth by Flesch. The degree of inhibition of melanin formation with skin extracts varies directly as the logarithm of the molar concentration of sulfhydryl (76). If the shaved skin of rabbits is exposed to ultraviolet radiation *in vivo*, a potent stimulus to melanin formation, there is an immediate decrease in sulfhydryl concentration in the skin of from 24 to 83 per cent (76). Epidermal extracts and also pure sulfhydryl compounds inhibit not only the effect of tyrosinase but also that of cupric ions on melanin formation, thus indicating that it is the copper moiety of the enzyme that is kept suppressed by the sulfhydryl groups (75).

Brilliant proof that tyrosinase exists in a partially inhibited state in human skin came with the work of Fitzpatrick, Becker, Lerner, and Montgomery (74), who, as previously mentioned, demonstrated histochemically the presence of tyrosinase in the epidermis after releasing it from its inhibited state by repeated ultraviolet irradiations. Fitzpatrick further showed that in benign pigmented lesions tyrosinase is present only in an inhibited state, while in many malignant melanomas the free enzyme can be directly demonstrated (73).

Recently it was also shown that hyperpigmented skin in postinflammatory condi-

tions has about half the sulfhydryl content of the surrounding skin and that vitiliginous skin has about twice as high a sulfhydryl content (291). At least two interpretations were suggested for the latter finding. Either the high sulfhydryl content prevents melanin formation by inhibiting tyrosinase activity, or sulfhydryl may accumulate secondarily because no melanin is being formed—a process which would use up sulfhydryl groups. Many of the intermediates in the melanin-forming chain of reactions are known to react with such groups.

From the preceding discussion it might appear that our knowledge about the normal regulation of melanin formation is complete. The following two points, however, have not yet been entirely reconciled with the sulfhydryl partial enzyme inhibition hypothesis. First, the long latent period of 1 week after ultraviolet irradiation of human skin required for release of tyrosinase activity is difficult to harmonize by the theory with the immediate drop in sulfhydryl content that has been observed in animals after such irradiation. Of course, a quantitative factor might be involved here, and possibly there is a delayed drop in sulfhydryl content as postinflammatory hyperkeratinization from such irradiations sets in. Second, and perhaps more crucial, is the repeated failure to date to show directly by histochemical methods that specific sulfhydryl poisons, such as mercurials, sodium arsenite, and iodoacetamide, can release inhibited tyrosinase activity in human skin (73, 171).

2. Competitive Inhibitors

To return to the outlined general categories of melanin-forming reaction inhibitors, the second classification to be considered is the group of competitive inhibitors. These are chemical compounds which inhibit enzyme reactions through uniting with the enzyme in competition with the substrate, thereby preventing the formation of an enzyme-substrate compound. Generally, true competitive inhibitors have two major characteristics: (1) in order to be effective, the inhibitor must be present in

much greater concentration than the natural substrate, and (2), by adding an excess of the natural substrate to the system, the inhibition can be reversed. Usually there is a close stereochemical relationship of the competitive inhibitor to the natural substrate.

N-acetyl tyrosine has clearly been shown to be a competitive inhibitor in the tyrosine-tyrosinase system, and it is probable that the other previously mentioned inhibitory analogues of tyrosine, such as 3-amino, 3-fluoro, and N-formyl tyrosines, also act in this way (163). In vivo tests with such inhibitors have not been reported so far. It is likely, however, that such tyrosine analogues are highly toxic, because they would be expected to interfere with other vital functions of this important amino acid.

3. Induction-Period-prolonging Factors

Among inhibitors that act by prolonging the induction period of the tyrosine-tyrosinase reaction are pH's above 7.5 (157). The detergent "Tween 20" is also believed to act in this way (157). Changes in the redox potential of the system also affect the length of the induction period, as has been previously pointed out. Increasing sodium chloride concentrations, which reportedly slow tyrosine to melanin oxidation by potato tyrosinase, also probably act in this manner.

4. Compounds Reacting with Melanin Precursors

Compounds reacting with intermediates in the melanin-forming reactions are of considerable interest. I undertook a study (170) of this group of inhibitors in a search for substances which would be taken up selectively and fixed at melanin-forming sites. Such substances, of course, could serve as keys by which selective destruction of melanin-forming cells might be possible in a fashion analogous to radioactive iodine destruction of thyroxine-producing cells. Substances combining with orthoquinones or orthodihydroxy melanin intermediates comprise this group of inhibitors. It must be

pointed out that none of this group of inhibitors has been shown to have *in vivo* activity.

Many aromatic amines or aniline derivatives (228, 157, 218, 181), i.e., compounds with an amino group attached to a benzene ring, react with the orthoquinone intermediates to form anilinoquinones. However, the inhibitory action of these aromatic amines as observed under actual experimental conditions is not quite so simple, as I pointed out after studies on oxygen uptake in mammalian tyrosinase-tyrosine systems with such amines added. Wide differences in oxygen-uptake inhibiting action of these compounds were noted. Some, such as ortho-aminobenzoic acid, failed to inhibit at all, whereas the isomeric para-aminobenzoic acid was a most potent inhibitor. Paraphenylene diamine also was a stronger inhibitor than orthophenylene diamine. There was, in addition, evidence that the latter prolonged the induction period for tyrosinase action and also that copper partially reversed its inhibitory effect.

It had been suggested in the past that the melanin-inhibiting action of molybdates in cattle might be due to the affinity of molybdenum for orthodihydroxy compounds (69). However, as will be discussed, it is more probable that molybdenum acts by interfering with copper metabolism.

5. Reducing Agents

Ascorbic acid is perhaps most important among inhibitors of the general class of reducing substances (218, 258, 280, 1, 257, 103). Although ascorbic acid enhances the transformation of tyrosine to dopa by ultraviolet radiation, it prevents further oxidation to dopa-quinone by its reducing action (242). It can also reduce oxidized dark melanin to its lighter reduced form (242). Hydroquinone, its derivatives, and analogous compounds also act as inhibitors *in vitro*, in part, at least, because of their reducing action. Ascorbic acid has been noted to decrease the pigmentation in Addison's disease (2, 302, 281) and on local injection in high concentration to prevent sun-tanning (300).

6. Incompletely Understood Inhibitors

Among inhibitors of unknown mode of action are pentachlorinated naphthalenes, hydroquinone and its monobenzyl ether, and *p*-hydroxypropiophenone. The depigmenting actions of the pentachlorinated naphthalenes and the monobenzyl ether of hydroquinone (*Agerite alba*) were first encountered in workers exposed industrially to these substances. The pentachlorinated naphthalenes are used as cable insulators. Cotter (47) reported many cases of dermatitis followed by leukoderma among workers handling this material. Several fatal cases of toxic liver disease also occurred among such workers. No attempts have been made thus far to analyze the mode of action of the compounds. In view of the dermatitis preceding the leukoderma, the latter may simply represent a type of postinflammatory depigmentation.

Leukoderma from monobenzyl ether of hydroquinone was noticed by Oliver *et al.* (214, 259) in the course of their investigation of occupational leukoderma in tannery workers wearing rubber gloves made of rubber containing *Agerite alba* as an antioxidant. Since then, numerous cases of leukoderma from contact with this compound have been recorded (15, 169, 270), and attempts have been made to use this agent as a topical bleaching agent in various melanodermic disturbances (72, 53). As is the case with the pentachlorinated naphthalenes, *Agerite alba* is a potent sensitizing agent, and contact dermatitis has frequently preceded the leukoderma. Some observers (15), in fact, suggest that leukoderma from this substance simply represents a type of postinflammatory depigmentation, with the preceding dermatitis in some cases being scarcely noticed. It is, of course, well known that leukoderma may follow very trivial inflammatory processes, especially in dark-skinned races.

Many attempts to analyze the mechanism by which this compound causes depigmentation have been made. Early work with patch tests indicated that an average of 27 days was required after initial contact

before leukoderma became evident and that in a majority of cases pigment returned only after many months (214). The depigmented areas, on histologic examination, contained no dopa-positive cells. Animal experiments and in vitro studies on this compound were first carried out by Peck and Sobotka (223). They were able to produce local depigmentation in guinea pigs with the substance but found it ineffective by oral administration.⁴ In vitro they noted that tyrosine or dopa oxidation in its presence did not proceed to melanin but rather led to the formation of a soluble red substance. Later it was found that this red substance is an oxidation product of monobenzyl ether of hydroquinone brought about by tyrosinase (153). I showed, further (170), that tyrosine oxidation by melanoma tyrosinase was stopped in the presence of this compound prior to the formation of indole derivatives; that the induction period of tyrosinase action was shortened; that it was not a true competitive inhibitor of tyrosine oxidation or a direct enzyme poison; and that it did not interfere with oxygen uptake in enzymatic dopa oxidation. Denton, Lerner, and Fitzpatrick (53) have confirmed these in vitro observations.

These in vitro experiments still leave unexplained the action of monobenzyl ether of hydroquinone in vivo, especially as regards the prolonged duration of the leukoderma after exposure to the substance is discontinued. This would either suggest some indirect antienzyme action or support the post-inflammatory depigmentation concept.

Hydroquinone by oral administration has been reported to cause depigmentation in cats, rats, and mice (213, 180), which is reversed after discontinuance of hydroquinone feeding. Rothman was not able (246) to confirm this finding in cats. The closely related parahydroxypropiophenone (53, 104) has also been reported to be capable of producing leukoderma in a fashion similar to

hydroquinone. Some pituitary-inhibiting action has been ascribed to this compound, which acts against pigmentation only via systemic administration.

The mechanisms by which nitrogen mustards (144) and ionizing radiations, such as X-rays (57, 41, 42), cause local depigmentation are also incompletely understood.

G. MELANURIA

In patients with metastatic melanoma it is well known that melanuria and, rarely, generalized melanosis may occur (154, 293, 51, 56, 21, 212, 288, 236). In melanuria, colorless or lightly colored melanin precursors are excreted in the urine, which, on oxidation, yield a dark precipitate. Early tests for melanuria were based on the addition of oxidizing agents, such as ferric chloride or bromine, to urine and looking for the appearance of a black precipitate.

Melanuria comes about because extensive melanotic tumors produce such great amounts of tyrosine oxidation products that some of them, like 5,6-dihydroxyindole, spill over into the general circulation and are excreted in the urine. Occasionally these circulating oxidation products of tyrosine may be oxidized to melanin in other tissues and thus account for the generalized intense melanosis that sometimes occurs.

Linnel and Raper (168) in 1935 reported that the melanogen in the urine of a patient with metastatic melanoma with melanuria was actually a simple derivative of 5,6-dihydroxyindole. Feeding of indoles not substituted in the pyrrole ring has been seen to cause true melanuria, whereas substituted indole compound feeding leads rather to the excretion of indoxyl and indican (30).

Rothman (245) in 1942 showed that indole, indican, and urinary melanogen could be differentiated on the basis of Herter's and Thormählen's tests for indole. The latter, incidentally, was found fifty times as sensitive for melanogen in urine as were the oxidation methods. Indican gave negative reactions to both tests, indole gave positive reactions to both, while the melanogen gave a negative Herter's test and a positive

4. Denton and Lerner, however, claim that depigmentation in animals can be produced by oral administration of this compound (53).

Thormählen test. White (299) has more recently devised a quantitative modification of the Thormählen procedure for accurately measuring the amount of melanogen excreted in the urine.

Early reports of melanuria occurring in

conditions other than metastatic melanoma (225, 110) are open to question because they are all based on simple oxidation tests for melanogen and many substances other than true melanogen can react positively to such tests.

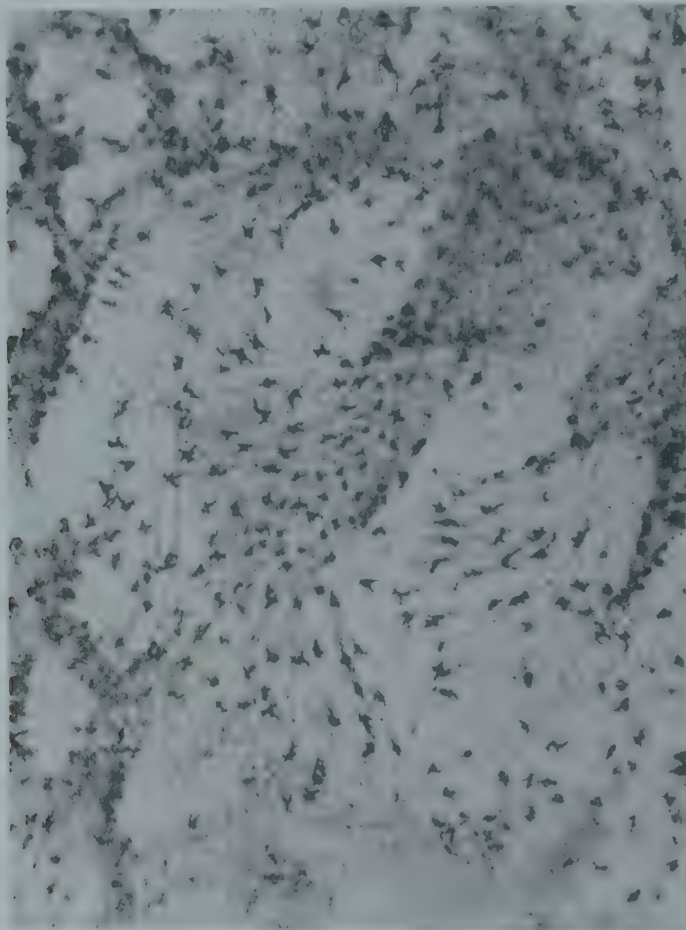


FIG. 6.—Separated epidermis from white human skin; dermal surface, showing nonpigmented melanocytes; dopa stain. From Becker, Fitzpatrick, and Montgomery (9). (Reproduced by permission of Dr. Becker and the American Medical Association.)

IV. BIOLOGY OF DENDRITIC MELANOCYTES

A. ANATOMICAL AND EMBRYOLOGICAL CONSIDERATIONS

In all vertebrates melanin formation is believed by most observers to take place primarily in specialized dendritic cells called "melanocytes."⁵ In human skin, melanocytes appear in haematoxylin-eosin-stained sections as regularly distributed clear cells in the basal layer of the epidermis (Fig. 7). Under special circumstances the dendritic nature and melanin-forming capacity of

these clear cells becomes apparent, and, furthermore, it can be shown that they interconnect via their dendrites to form a semisyncytium (18, 17, 9). These features are especially evident in skin that has been ex-

5. The melanocyte was defined as the normal mammalian melanin-forming cell at a meeting on nomenclature during the Third Conference on the Biology of Normal and Atypical Pigment Cell Growth in November, 1951, in New York (96). Previously these cells were commonly called "melanoblasts."

posed to pigmentogenic stimuli, such as ionizing radiations, and if staining techniques like the dopa procedure or acid gold chloride are used on sections cut parallel to the skin surface (221). Apparently, there is more branching of these cells in a tangential, than in a perpendicular, direction (Fig. 6). That the clear cells or melanocytes are hydrated to a greater degree than their neighboring basal cells is indicated by their greater susceptibility to destruction by freezing (283) and also by the fact that their charac-

laden with granules of melanin, and such granules may be scattered upward throughout the entire epidermis and may even fill macrophages in the upper corium.

Although most observers agree that dopa-positive clear cells or dendritic melanocytes are major sites of active melanin formation, the neural-crest origin of these cells in man and explanations for the appearance of the majority of melanin granules in the palisade basal cells have been subjects of dispute. Early observers (221) considered the dopa-

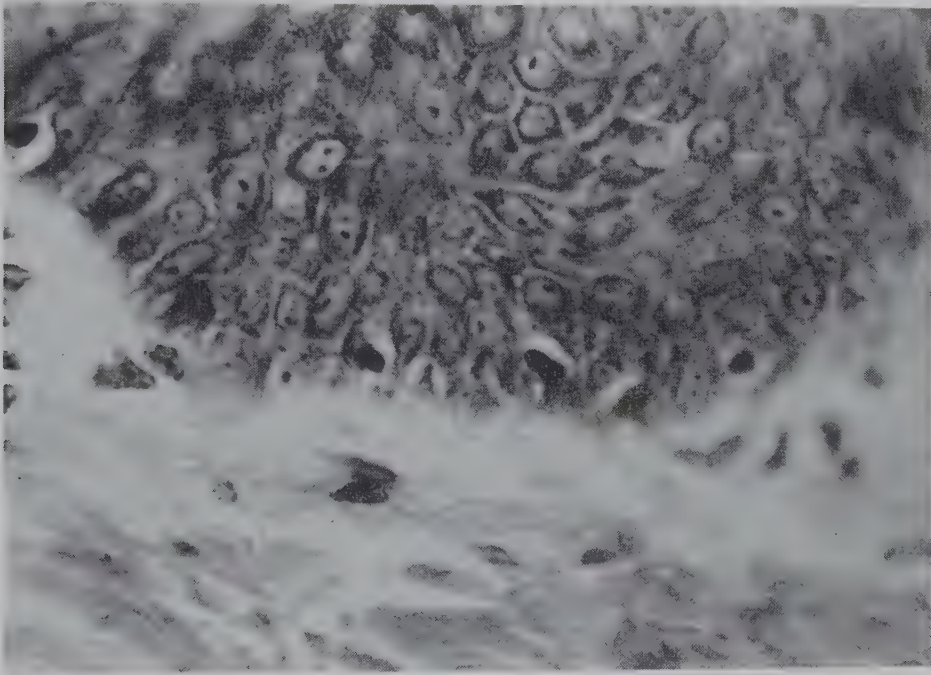


FIG. 7.—Section of human skin, showing clear cells in the basal layer

teristic clear appearance is chiefly the result of cytoplasmic pseudo-vacuole formation, an artifact caused by shrinkage of the cytoplasm during the process of fixation. Possibly the increased selective destructibility of melanocytes by the very short-lasting intense heat associated with atomic-bomb flash burns (166) can also be related to the increased hydration of these cells.

In routine microscopic cross-sections of human skin, most melanin granules ordinarily appear as if clustered above the nuclei of the palisade basal cells rather than in the dopa-positive melanocytes. In deeply pigmented skin all the basal cells appear heavily

positive dendritic cells to be merely basal cells in a special phase of active pigmentogenesis and that, under suitable stimuli, basal cells which ordinarily were only weakly dopa-positive, if at all, became strongly dopa-positive, transformed into dendritic cells, and actively produced melanin. By this theory, all basal cells could potentially form melanin, and there was no particular problem involved in explaining the presence of large amounts of pigment in the basal cells. Some early observers (3, 24) even denied the reality of the dendritic processes, merely considering them to represent streams of free intercellular pigment. Recent

tissue-culture studies, however, of fish, mouse, and human melanomas have clearly proved the reality of the dendritic processes of melanocytes (98) (Fig. 8).

As later careful embryologic ablation and transplantation experiments upheld the neural-crest origin of melanin-forming cells in amphibians and birds and strongly supported a similar derivation in mice (60, 233, 123), further attempts to account for the distribution of melanin were made. Masson (188) postulated that the basal layer consisted of two distinct types of cells—the clear cells of neuroectodermal origin and the

tion, in that the product formed is passed into other cells rather than to the exterior or into vascular channels. In strong support of the cytotrine theory, Masson cited observations that when cancer cells, such as those of mammary or even of digestive-tract origin, invade the epidermis, they occasionally seem to become stuffed with melanin granules. Sometimes even deeper portions of the tumor and its stroma become similarly melanized, and this he presented as evidence for a potential motile property of melanocytes.

S. W. Becker, Jr., *et al.* (9) have recently brought forth evidence challenging even the

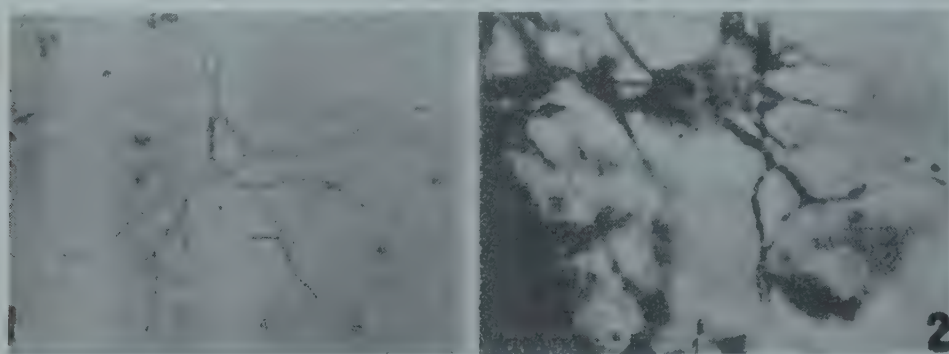


FIG. 8.—1, photograph of a small-type melanoblast from a living culture of human melanoma. The melanin granules are sparsely distributed in the dendrites. 2, Large-type melanoblast (dopa-treated) from a culture of Harding-Passey mouse melanoma. The branching dendrites are filled with melanin granules, giving them a varicose appearance. From Grand and Cameron (98). (Reproduced by permission of the New York Academy of Sciences.)

regular palisade epidermal basal cells. He further revived and extended earlier concepts. In these concepts it was postulated that the dendritic cells or melanocytes were the sole source of melanin granules and that these cells inoculated melanin granules, via their dendrites, into surrounding cells with which they came into contact. Earlier workers cited by Masson observed convincing evidence for such an inoculation function, particularly in developing bird feathers, in fish and amphibian epidermis, and in the human hair root. Stearner (273), in freshly prepared frog skin, has even directly observed such transfer of pigment granules from melanocytes into epidermal cells. Masson called this cellular melanin secretory process “cytotrine,” because it differs from both exocrine and endocrine types of secre-

fundamental observation that melanin granules are situated within basal cells. They claim that this appearance is illusory and that the pigment is really in the ends of dendritic cell processes that essentially form a sheath surrounding each basal cell.

B. NATURE OF MELANIN GRANULES

Melanin granules, as they occur in mammalian pigment cells, are specific structures with some rather unusual attributes instead of being merely amorphous chunks of melanin. Among several lines of evidence which support this view are results of electron microscopy of melanin granules obtained by differential centrifugation from mouse melanomas, beef eyes, and colored human skin (185). In all instances these melanin granules were revealed to be formed ele-

ments having characteristic uniform rounded shapes for each particular source, and to be approximately $0.3\ \mu$ in diameter (Fig. 9). In addition, naturally occurring mammalian melanin can be shown chemically to be combined with a protein, a pseudo-globulin with antigenic properties and with a minimum

molecular weight of 20,000 (100). When such a melanin pseudo-globulin, which can be extracted from mouse melanomas, is progressively digested by pancreatin, the sulfur content of the protein remaining attached to the melanin moiety increases. This has been interpreted to mean that the



FIG. 9. — Electron micrograph of melanin granules from the Harding-Passey mouse melanoma obtained by chromatography. (Gold shadowed at an angle tangent of approximately one-third. Particles $0.3\text{--}0.4\ \mu$ in diameter.) From V. Riley, G. Hobby, and D. Burk, *Oxidizing enzymes of mouse melanomas: their inhibition, enhancement and chromatographic separation*. In: *Pigment cell growth* (New York: Academic Press, Inc., 1953), pp. 231–66. (Reproduced by permission of Dr. Riley and Academic Press, Inc.)

melanin is attached to the protein by sulfur linkages (100).

Furthermore, Stary (272) has extensively studied another melanoprotein obtained by treating dark hair with dilute sodium sulfide. This material remains indigestible to trypsin, whereas a leukokeratin extracted in the process becomes trypsin-digestible. This work indicates that the resistance of this type of melanoprotein to trypsin is not based on the presence of disulfide bonds (p. 356) but rather on the presence of melanin which may cross-link polypeptide chains in similar fashion. This cross-linking function of melanin or its precursors might account for the well-known greater resistance of deeply pigmented skin to external irritants.

As further evidence of their specific structural nature, melanin granules isolated from mouse melanomas have been shown to have a considerable nucleic acid content (307). On alkaline hydrolysis, such granules yield solutions which contain organic phosphorus and pentose and which show strong ultraviolet light absorption at 2580 Å (307). Several enzyme activities have also been demonstrated on such granules, which have been concentrated by chromatographic adsorption of melanoma homogenates on celite columns (235), with subsequent elution by saline solutions. These enzyme activities include the aerobic cytochrome oxidase and succinic oxidase systems and anaerobic glycolytic activities. Besides these banal enzymes, the unique copper-containing enzyme, tyrosinase (or dopa oxidase), is present, which is responsible for the melanization of the granules. On the basis of these morphological, chemical, and enzymatic observations, melanin granules have been considered to be mitochondria, which are different from other mitochondria only in possessing the additional pigmentogenic enzyme, tyrosinase, which brings about the oxidation of tyrosine to melanin.

C. PHENOMENON OF PIGMENT SPREAD

If melanin granules are truly mitochondria, they should exhibit other outstanding properties of these fundamental cellular

particles, namely, self-duplication and an independent hereditary system. The terms "plasma-genes" or "chondriogenes" have been applied to hereditary factors residing in mitochondria. That melanin granules have these additional properties is illustrated particularly well by the phenomenon of pigment spread. This phenomenon has been known for more than fifty years but has been especially studied in the last fifteen years by Lewin and Peck (165) and by Billingham and Medawar (19, 20). It is seen in black and white animals, such as recessively spotted guinea pigs, when black skin from a black patch is transplanted into a white area. Under these conditions there results a slow extension or spread of the pigment into the surrounding white area from the dark graft. Similarly, if a white graft is placed in black skin, pigment spreads from the peripheral black area into the white area. Billingham and Medawar recently interpreted this phenomenon as an "infective" spread of mitochondrial self-duplicating pigment granules into colorless melanocytes in the white-skin areas via the "cytocrine" activity of the melanocytes. They have demonstrated, by acid gold chloride staining, the anatomic presence of a partially syncytial web of such nonpigmented dendritic cells in the white areas of skin and, furthermore, that it is these cells which become "infected" with melanin granules and that it is within them that these granules multiply and, in turn, spread farther to "infect" adjacent "white melanophores." That the pigment involved in pigment spread is the result of a self-duplication process is indicated by experiments in which the antigenically foreign nature of this pigment can be shown. In these experiments, pigment spread is induced by a special transplantation technic, using a suspension of black-skin basal layer from one animal which is then inoculated into the white skin of another animal. Under these circumstances the new pigment formed can be abolished by transplanting normal skin from the original donor animal to the recipient in which the pigment spread had occurred. This abolition

of the pigment, according to Billingham and Medawar, is the result of the active immunization of the recipient to the donor skin antigen. The new pigment formed, being antigenically of the donor type, is believed to be susceptible to destruction when active immunity induced in the recipient becomes sufficiently great.

Whether the phenomenon of pigment spread occurs in human skin is not entirely clear. Peck (222) states that hyperpigmentation has been observed in the vicinity of human skin grafts from this mechanism and that it can lead to serious cosmetic defects. On the other hand, Haxthausen (120) reported on transplantation experiments in vitiligo and congenital lentigo where the phenomenon did not occur. In the case of

lentigo, the grafts of pigmented skin to normal skin and of normal skin to lentigo-involved areas both remained completely unchanged and did not cause alterations in the surrounding skin. Furthermore, in vitiligo the transplants, whether from pigmented to nonpigmented skin or vice versa, always took on the characteristics of the area in which the graft was placed. Thus normally pigmented skin became depigmented when placed in a vitiliginous area and depigmented skin became repigmented when placed in a normal area. These observations of Haxthausen in vitiligo were confirmed by similar experiments done by others (45). Haxthausen interpreted his findings as suggestive of a neurotrophic basis for vitiligo.

V. NUTRITIONAL FACTORS IN MELANIN FORMATION

Nutritional deficiencies of several sorts have been associated with either deficient or excessive melanin pigment formation in several animal species as well as in man. Frost (83) reviewed this subject thoroughly in 1948. The nutritional factors influencing melanin formation can be classified into three major groups: (1) trace metals, (2) amino acids, and (3) vitamins.

A. TRACE METALS

Among trace metals, copper is of prime importance, as was brought to light by Keil and Nelson in 1931 (141) while they were studying copper deficiency in rats produced by feeding milk diets. Besides their developing anemia, the hair of these animals, as it grew, became gray. After 2 months of supplementation of this diet with only traces of copper, pigment again was formed in the growing hairs. This work in rats has since been confirmed, and copper-deficiency graying has also been noted in rabbits (268), cats (97), and cattle (267). In rabbits fed copper-deficient milk diets supplemented with iron, it is of interest that not only achromotrichia but hair loss and scaly dermatosis also developed, which could be reversed by copper administration. The diminished melanin

pigmentation in copper deficiency is most easily understood in terms of the necessity of copper for tyrosinase activity. However, a recent claim that copper-deficiency depigmentation in mice can be reversed with pantothenic acid (264) adds a confusing element to the picture. This could be understood, however, in terms of the suggestion that pantothenic acid might be involved in the linkage of copper to tyrosinase (264). This suggestion fits in with the observation of Hundley and Ing (126) that copper can accumulate in the skin of rats showing graying from pantothenic acid deficiency without activating melanin formation.

It is more difficult to interpret the reported loss of pigment in rats in acute zinc deficiency, which is reversible by minute amounts of zinc added to the diet (274). In this connection, however, it is interesting to note that zinc is present in considerable amounts in hair (175) and also that there have been recent discoveries of relatively large amounts of zinc in association with melanin in the pigment fraction of mammalian eye tissues (32). In fact, ten times as much zinc as copper was found. Calcium, magnesium, and barium were also present. The concentration of these elements in the

melanin pigment fraction suggests that the pigment is a potent chemical chelating agent (organic metal complexing agent).

The depigmentation associated with diarrhea and emaciation in cattle, reported by Muir (203) to be caused by chronic excessive molybdenum intake, is probably related to an interference by molybdenum with copper metabolism, because adding copper to the diet cures the syndrome. Comar (44) has further shown by radioactive isotope studies that molybdenum decreases the storage of copper by the liver.

Thallium has also been reported to cause graying of black rats (102).

Some trace metals, when present in excessive amounts in the skin, are often associated with increased melanin formation. These include iron, silver, gold, bismuth, and arsenic (216). Most of these gain access to the body in excessive amounts via medicaments. It has been postulated that these metals cause melanosis by binding tissue sulfhydryl groups, which normally keep tyrosinase in a partially inhibited state (157), as has already been pointed out. The locally precipitated metals or metal compounds also contribute to the dark color of the skin.

B. AMINO ACIDS

The amino acid and protein requirements for normal melanin formation have not been very thoroughly studied. Amino acids, however, are needed for melanin formation, not only as a tyrosine substrate for enzyme action but also as necessary components of enzyme protein. Hartwell (119) in 1923 first suggested that protein and especially tyrosine or tryptophane deficiency might play a role in the graying of rats on a diet of bread, milk, and kitchen scraps which could be corrected by "food casein." These early experiments, however, are not reported in sufficient detail to render it certain that mineral or vitamin deficiency corrections may not have been involved.

Another facet in the amino acid picture is the report that cystine added to the diet of pantothenic acid- and cystine-deficient rats

augments the return of pigment when calcium pantothenate is given (220). That lysine may also be implicated is suggested by the report of deficient feather pigmentation in bronze poult on a high vegetable protein diet, which was reversed by adding lysine (82). That tyrosine deficiency may result in deficient melanin production has been hypothesized by Lerner and Fitzpatrick (157) to explain the light skins and deficient sun-tanning ability of individuals with phenylpyruvic oligophrenia. In this rare, recessively inherited disease, a biochemical metabolic anomaly exists so that normal hydroxylation of phenylalanine to tyrosine cannot occur. These individuals are therefore entirely dependent on dietary tyrosine intake for this amino acid. They excrete phenylpyruvic acid in the urine and have high levels of phenylalanine and low levels of tyrosine in the blood (133).

A number of severe nutritional deficiency states have been reported to be associated with graying of hair in children of colored races from Malaya (208), Costa Rica (224), and South Africa (303). These have been reported under various names, such as "kwashiorkor" (5), "infantile pellagra" (91, 287), and "malignant malnutrition" (208, 92). Protein-deficiency edema generally accompanies these syndromes, which are often fatal if untreated but which are readily cured by adequate diets. Whether the achromotrichia results from vitamin, protein, or mineral deficiencies has not yet been clarified. Vitamins alone, however, fail to cure the syndrome.

This particular deficiency disease is the one instance where, regularly, gray hair in man can be reversed by nutritional factors. In contrast, there is a vast array, as will be pointed out, of animal experiments in which such factors can be demonstrated. Certainly, the ordinary graying of human hair has never been consistently reversed by dietary manipulation, including administration of all known vitamins. Administration to man of para-aminobenzoic acid in huge doses, however, has been reported to cause, on occasion, return of pigment to gray hair (311).

C. VITAMINS

As concerns vitamins, two independent reports (198, 172, 173) appeared in 1938, noting the graying of rats maintained on synthetic diets deficient in only the filtrate factors of the vitamin B complex, which are factors other than thiamine, riboflavin, and pyridoxine and are not adsorbed on Fuller's earth. The addition of these filtrate factors restored normal pigmentation. This phenomenon was also observed in mice (105, 180), dogs (199, 84), guinea pigs (199), foxes (200), and chicks (101). It was also shown that avitaminotic graying may become irreversible (possibly from permanent damage to melanocytes) if the deficient diet is given over a sufficiently long time (174). In the early 1940's, a number of workers studied the antiachromotrichial properties of the various components of the filtrate factor and reported varying results, which implicated to differing degrees pantothenic acid (105, 101, 107, 106, 210, 289, 55, 304, 86, 64, 195, 177), para-aminobenzoic acid (180, 6), biotin (277, 65), and, at that time, some still other unknown factor present in liver and yeast. Pantothenic acid, however, seemed to be the chief factor involved. Frost and his co-workers (85) in 1946 helped clarify the problem by showing that synthetic pteroylglutamic acid (folic acid) completely restored pigment in chicks depigmented on synthetic diets containing all other known dietary factors, including pantothenic acid, biotin, copper, zinc, para-aminobenzoic acid, choline, cystine, and inositol. Lillie and Briggs (167) later confirmed this work. Finally, Wright and Welch (308) further clarified the problem. They noted that liver storage of pantothenic acid was aided by simultaneous administration of folic acid concentrates and biotin when given to succinylsulfathiazole-fed rats which had become gray. This would indicate that the

utilization of pantothenic acid is in some way dependent on the presence of folic acid.

The following working hypothesis can perhaps best summarize the observations in this complex field. Pantothenic acid can be considered the chief anti-achromotrichial vitamin. In the absence of folic acid, biotin, and possibly other factors (such as choline, inositol, and cystine), either pantothenic acid cannot be synthesized because of ineffectual bacterial flora in the intestines, or the tissues cannot utilize it properly. Para-aminobenzoic acid could further act indirectly by being essential for folic acid synthesis.

Deficiencies of vitamins A (202), C (46), and niacin (118) have all been reported to lead to increased melanin formation. Thus in pellagra from niacin deficiency, melanosis characteristically follows the acute inflammatory lesions appearing on exposed areas. Photosensitization undoubtedly plays an important role in these lesions, and the melanosis is probably a type of postinflammatory hyperpigmentation. The melanosis in vitamin A deficiency occurs at the sites of the follicular hyperkeratotic papules. It has been suggested (157) that the local reduction in sulfhydryl groups accompanying increased keratinization may serve to release at these points the normal sulfhydryl inhibition of tyrosinase. The melanosis occasionally associated with scurvy can also probably be accounted for on the basis of release of sulfhydryl inhibition. Ascorbic acid is a major factor in maintaining sulfhydryl levels in tissue and blood. It is also possible that iron, deposited in the skin as a result of the cutaneous hemorrhages of scurvy, may bind sulfhydryl inhibitors of tyrosinase. Such a mechanism has been proposed for the excess melanin formation in hemochromatosis (157).

VI. ENDOCRINE FACTORS IN MELANIN FORMATION

The relationships between hormonal factors and melanin formation have been widely studied in both lower animals and man.

Pituitary, thyroid, adrenal, and gonadal hormones have all been shown to influence melanin pigmentation in living animals. In

vitro biochemical experiments on melanin formation with these hormonal factors, however, have been very meager.

A. PITUITARY

The pituitary gland exerts its effect on pigmentation either by influencing other endocrine glands or through the melanocyte-stimulating hormone (MSH, melanophore hormone, intermedin). In cold-blooded vertebrates both neural and humoral factors can bring about rapid changes in coloration in response to many stimuli. The humoral control is brought about by the direct action of MSH on chromatophores in the skin to cause dispersion of pigment granules within them. If the hypophysis is removed in an animal like the frog, the skin pales because the pigment granules cluster together in the chromatophores. If into such a hypophysectomized animal injections of pituitary extracts from vertebrates of all types, including mammals, are given, the chromatophores rapidly appear to expand because of pigment dispersion, and the skin darkens.⁶ Even in excised pieces of frog skin the chromatophores respond in similar fashion to pituitary preparations (309). The response of excised frog skin has been used with reflectance spectrophotometry as a sensitive assay method for MSH (158).

Some evidence has been brought forth in amphibians that prolonged melanophore expansion results in an absolute increase in amount of dark melanin pigment in the skin (50, 49, 292). Freiden and Bozer (81) also noted an increase in melanin content of about 40 per cent in frog skin after 8 weeks of MSH injections.

Although MSH is clearly present in mammalian pituitaries (125), no rapid expansion of mammalian melanocytes can be brought about by it. Nevertheless, there have been

6. Photoelectric measurement and microscopic observation of melanophore responses in such frogs have been used for sensitive MSH assay purposes (Eric Thing, Melanophore reaction and adrenocorticotrophic hormone. I. Comparison of methods based on photoelectric measurements and microscopic observations of the melanophores, *Acta. Endocrinol.*, 10:295-319, 1952).

suggestions that this hormone may exert a stimulating influence on mammalian melanin formation. Thus, Calkins (quoted in 157) hypothesized that the melanosis in Addison's disease comes about by release of adrenal inhibition of pituitary MSH output, the increased blood levels of MSH then being the direct cause of the increased melanin synthesis. This hypothesis is supported by the observation that secondary adrenal cortical insufficiency from hypopituitarism is usually accompanied by decreased pigmentation (263). Furthermore, it has been suggested that the Addisonian type of melanosis occasionally accompanying the administration of ACTH in man may result from the high MSH activity of such preparations (157). Such ACTH melanosis, however, has also been suggested as resulting from diminished skin sulfhydryl content, an effect likely to occur in view of the reduction in blood glutathione accompanying administration of either this hormone preparation or cortisone (284). Cortisone administration has also been stated to result in melanosis, although much less frequently than is the case with ACTH. In my experience cortisone is not pigmentogenic, and some question exists about the phenomenon in the reported cases, because definite statements are not available as to whether ACTH had not also been previously given to these patients. Of course, the reduced skin sulfhydryl theory would be applicable to explain melanosis from cortisone if it really occurs. However, Hall, McCracken, and Thorn (111), on the basis of reflectance spectrophotometric studies, find that cortisone generally causes a decrease in skin melanin content, whereas ACTH causes an increase.

MSH is a relatively heat-stable, small polypeptide hormone produced in the intermediate (and/or anterior) lobe of the pituitary gland.⁷ Whether it is made independently from ACTH is much debated (89, 137, 229, 278). Separation of MSH from ACTH without loss of either activity has not been

7. In the whale, which has no intermediate lobe, Geiling and his co-workers (88) showed that the anterior lobe of the pituitary produces this hormone.

accomplished and even the most highly purified ACTH preparations have high MSH activity. ACTH activity, however, can, in ACTH preparations, be destroyed without destroying melanophore activity by boiling. Recently preparations of extremely great melanophore-expanding potency have become available, such that as little as 10^{-10} gm. is said to cause darkening of hypophysectomized frogs (158). The dilutions required to achieve these low concentrations, however, are difficult to evaluate because complicating adsorption effects easily lead to errors; but even if the figure of 10^{-10} is not strictly correct, there can be no doubt about the very high potency of these preparations.

Whether MSH can act in mammals by causing a very slow dispersion of melanin granules within melanocytes is still an open question. Certainly, a great many pigmentogenic stimuli do make the dendritic processes of these cells more readily visible as a result of their filling with melanin granules. Whether this filling of dendrites with melanin granules results solely from multiplication of such granules is not known, and it is distinctly possible that granule dispersion may also play a role.

Another suggestion as to a possible mechanism by which MSH might control mammalian coloration has been that this hormone causes increased serum copper levels, which, in turn, activate skin tyrosinase (158). Such increased copper levels have been reported to be associated with Riehl's melanosis (240, 219).

Fostvedt (80), moreover, suggested a direct tyrosinase-stimulating action of MSH on the basis of *in vitro* experiments on tyrosine-tyrosinase systems in the Warburg respirometer. Attempts to confirm this work, however, have not been successful (158, 171).

Although these varied theories for MSH stimulation of pigmentation in mammals have been proposed, the simple fact of such stimulation has only recently been proved. In man injections of very huge doses of purified highly potent MSH preparations have produced generalized melanosis and

stimulated the appearance of flat pigmented nevi.⁸ In rabbits there has been a reported failure of this hormone to influence pigmentation when slowly absorbed from implanted collodion sacs (197). Rather early reports of the beneficial effect (204) of local intermedin injections in vitiligo have not been confirmed. It would be of interest to repeat these latter experiments with the potent preparations of intermedin now available.

B. THYROID

In lower vertebrates thyroid hormone is important in maintaining normal melanin formation. Thus tadpoles fail to develop pigment in its absence (176); and Trinkhaus has shown that thyroxine in the adult brown Leghorn chicken enhances formation of black pigmentation, whereas in its absence a red pigment is formed (286). In rabbits Robert (238) found that intradermal injections of thyroxine caused locally increased pigmentation.

In man hyperthyroidism is frequently accompanied by increased pigmentation, while myxedematous patients are usually pale (132). On the other hand, vitiligo is frequently associated with hyperthyroidism (132). A similar finding has been reported in thyrotoxic chicks (108). Maybe this association is due to an increased tendency toward pigmentation in hyperthyroidism. It is known that melanosis is often accompanied by spotted depigmentation, e.g., in Addison's disease and arsenical leukomelanoderma.

In any case, it is difficult to interpret these thyroid effects on pigmentation. Most simply, one might speculate that thyroxine, as a general stimulator of oxidative processes, might also enhance the oxidation of tyrosine to melanin. Another interpretation along the lines of the sulfhydryl-inhibited tyrosinase theory has been that the hyperpigmentation simply accompanies a general

8. A. B. Lerner, K. Shizume, and T. B. Fitzpatrick, On the mechanism of melanin pigmentation in endocrine disorders, *J. Invest. Dermat.*, 21:337-38, 1953.

reduction in body sulfhydryl groups that has been reported in hyperthyroidism (157).

It might be of interest here to call attention to some very general similarities between the enzyme systems involved in the synthesis of thyroxine and of melanin. Both, for example, (1) utilize tyrosine as a substrate, (2) are inhibited by copper-binding agents, (3) are regulated in some fashion by pituitary tropic hormones, and (4) lead to an end-product which is normally bound to protein.

C. ADRENAL

Adrenal hormones play a very important role in the regulation of melanin formation. This is well illustrated by the striking melanosis characteristic of Addison's disease in man, produced by removal of the adrenal cortex either by disease or by surgery. In this disease diffuse melanosis slowly develops and becomes most intense over areas normally more deeply pigmented, such as the axillae, perianal area, areolae, exposed parts of the face and extremities, and pressure points. It is to be noted that many of these predilectional areas for the melanosis tend to be the relatively warm areas of the skin, and this locally increased temperature may be an additional factor accelerating the melanin-forming reactions in these sites both normally and in melanotic conditions (157). Although in most animal experiments no pigmentary changes follow adrenalectomy, in rats with achromotrichia induced by diets deficient in the filtrate factor, adrenalectomy more than restores pigmentation, and this pigment-restorative effect of adrenalectomy can be counteracted by desoxycorticosterone (230, 39, 271). In tissue cultures of embryonic chick skin explants, Hamilton (112) noted an inhibitory action of desoxycorticosterone on melanophore development. More recently Whitaker and Baker (298) also report that local application of adrenal cortical hormones causes loss of pigment in rat hair. Thus it is probable that the adrenal cortical hormones can exert an inhibitory effect on melanin formation in some rather direct fashion. Possible

indirect mechanisms for adrenal inhibition of melanin formation (via melanophore hormone restriction) have already been discussed (p. 544). Release of sulfhydryl tyrosinase inhibition, as was also mentioned, is perhaps another good partial explanation for Addisonian melanosis. In fact, Rivoire (237) in 1935 administered cysteine together with saline to Addisonian patients and noted marked reduction of melanosis. This observation had previously also been made by Léobardy and Labesse (155) in another Addisonian patient.

In the past many theories were proposed to explain Addisonian melanosis, and these will be discussed briefly, mainly for their historical interest. Sodeman (269) has reviewed these various theories. An early proposal championed by Bloch and Löffler (26) was based on the fact that epinephrine and melanin originate from the same precursor, tyrosine, and that if the adrenals do not utilize it for epinephrine synthesis, this substrate accumulates, to be converted to melanin. Obviously, this theory is deficient because of well-known cases of pigmentation in Addison's disease in which the adrenal medullae are intact. Another suggestion was that ascorbic acid, normally stored in large amounts in the adrenals, is destroyed and hence leads to a reduction in tissue ascorbic acid, which is a known inhibitor of melanin formation (281). This theory is difficult to support because ascorbic acid fails to reverse Addisonian melanosis except occasionally, when huge unphysiological doses (500 mg. or more daily) are used (244, 302). Another suggestion by Lea (151) was that the drop in blood sodium chloride may be the important factor involved. Experimentally he was able to show that in the presence of ascorbic acid the tyrosinase-tyrosine reaction proceeded faster at 0.5 per cent salt concentration than at 0.9 per cent. The former salt concentration, however, represents an unphysiological range, even for Addison's disease.

An interesting observation recently is that in patients in whom both adrenals have been surgically removed for treatment of

malignant diseases, gross melanosis does not develop while they are kept on maintenance doses of cortisone (13). If, however, desoxycorticosterone is added along with the cortisone, intense Addisonian-type melanosis does appear. No explanation is available to account for this latter unusual finding.

Most recently nor-epinephrine and, to a lesser degree, epinephrine have also been shown to affect melanocytes directly by blocking the melanophore-expanding effect of MSH in excised frog skin (158). Ergotamine abolishes these blocking effects. These observations confirm the much earlier claims that, in lower vertebrates, sympathetic nerve impulses cause contraction of melanophores (see p. 549).

D. GONADAL

Gonadal hormones, in general, promote melanin pigmentation both in man and in lower animals. It is well known that male eunuchs have pale skins and do not tan readily. Hamilton *et al.* (115) observed an additional interesting phenomenon in a male eunuch who had been exposed to the sun without developing pigmentation. Five months later, after administration of testosterone, strong pigmentation developed in the previously sun-exposed areas. Later he (113) reported similar observations in two postmenopausal and three castrate women. Pigmentation in these women developed after estrogen or androgen administration in areas exposed to the sun 2 months previously. Jodar (136) also reported a similar occurrence in a child who developed pigmentation in areas previously exposed to ultraviolet light irradiation after receiving a blood transfusion from the mother.

In 1932 Bloch and his co-workers (28) made ovarian implants into castrate male guinea pigs and noted that hyperpigmentation developed in the nipples, areolae, and about the genitalia. Davis, Boynton, Ferguson, and Rothman (48) noted this early work and studied the phenomenon further. They found that local application of estrogen to one nipple alone could induce purely local increased melanin formation, without

systemic estrogenic effects (Fig. 10). Thus it was rather conclusively demonstrated that this hormone can directly stimulate at least some melanocytes to increased melanin formation. Recently this work in guinea pigs was confirmed (297). W. Jadassohn *et al.* (129) even worked out a special "nipple test" based on the local pigmentogenic effect of estrogens. Davis *et al.* (48) further administered estrogen to young women with primary amenorrhea and noted the development of a striking persistent melanosis of the nipples, areolae, linea alba, and genitalia. Such a pigmentogenic effect from estrogen could not, however, be obtained in postmenopausal women. These workers hypothesized that increased gonadotrophins in the latter group might inhibit the estrogenic effect, and they were able to show in the castrate guinea pig that estrogen-induced nipple pigmentation could be inhibited by concomitant administration of chorionic gonadotrophin, but curiously not by pregnant-mare-serum gonadotrophin. It is difficult to see how such gonadotrophic inhibition could actually operate in the postmenopausal group given stilbestrol, in view of the inhibiting action that stilbestrol eventually has on gonadotrophin output. It would thus seem that a reduction in target organ sensitivity to estrogen with age might better explain the inability of estrogen to induce nipple pigmentation in these older women. The unusual chorionic gonadotrophin inhibition in the guinea pig, however, has yet to be explained.

The local hyperpigmentations developing in pregnant women are, without doubt, estrogenic effects, the melanocytes about the nipples, areolae, linea alba, and genitalia being specifically more susceptible to stimulation by greatly increased amounts of circulating estrogenic hormones. At times melanocytes elsewhere may also respond similarly to estrogen, viz., the chloasma of pregnancy and the darkening of pigmented nevi. Sunshine and estrogen may act synergistically in producing the chloasma of pregnancy, as was shown by Jesionek (134). He covered half the face of a woman who had

had chloasma in a previous pregnancy with light-impermeable material. Chloasma developed during the pregnancy only on the uncovered side.

Estrogens have been implicated in still other ways in pigment formation. Westersfeld (295) in 1940 found that natural estrogens having active phenolic groups could be oxidized by tyrosinase. Graubard and Pin-

oxidized and can no longer inhibit tyrosine oxidation. These experiments, if confirmed, suggest that the mechanism by which estrogen favors melanin formation by direct local action is by release of normal sulfhydryl inhibition of tyrosinase. The experiments of Figge and Allen have, however, been open to criticism because no controls were done to determine the action of their crude tyro-



FIG. 10.—Guinea pig in which nipple and areola (A) were treated by topical applications of diethylstilbestrol in sesame oil, while nipple and areola (B) were used as a control and received topical applications of sesame oil only. The enlargement and hyperpigmentation of A demonstrates the direct action of estrogens on the melanocytic cells without interference of general systemic effects. From Davis *et al.* (48). (Reproduced by permission of Dr. Davis and the Journal of Clinical Endocrinology.)

cus (99) confirmed this finding with potato tyrosinase and noted that the oxidation of estrogens led to red-colored quinone-type end-products without estrogenic activity. Figge and Allen (71) further noted that estrone can overcome glutathione inhibition of tyrosinase oxidation of tyrosine by acting as a more easily oxidizable substrate than tyrosine. The quinone formed by this process from estrogen then oxidizes reduced glutathione, until the latter is completely

sinase preparation on estrone alone. It is likely that their crude potato enzyme contained estrinase in addition to tyrosinase. Forbes's (78) observation of a reddish hair pigment developing in albino rats given large doses of estradiol can be interpreted to lend support to the concept that estrogens in mammals can be oxidized in some fashion to pigment. Forbes also reported darkening of the hair in male and female rats after implantation of pellets of androgens or estro-

gens. In tissue cultures of fowl skin, Hamilton (112) also noted that androgens and estrogens promote melanophore differentiation.

Like estrogens, androgens, besides having some general stimulating effect on pigment formation, can directly stimulate specific melanocytes in some species to form large amounts of melanin. The melanocytes of the bill of the sparrow (226); the lores, tongue, and floor and roof of the mouth of the night heron (211); the dorsolumbar spots of the hamster (149); and the scrotum of the thirteen-lined ground squirrel (294) respond in

such specific fashion to androgen stimulation, whether locally applied or systemically given.

Hamilton (114), on studying this effect of locally applied androgen in the hamster, further discovered that these particular melanocytes, in some unexplained fashion, can sum up the effects of small fractions of a total effective dose when these are applied in subthreshold amounts over intervals as long as 3 weeks.

In excised frog skin progesterone and pregnanetrione have had some direct melanophore-expanding activity (158).

VII. NEURAL FACTORS IN MELANIN FORMATION

That neural factors influence melanin formation is not surprising, in view of the close association embryologically between nervous tissue and melanin-forming cells. Furthermore, good evidence has been brought forth in urodeles that melanin-forming cells of the eye under appropriate conditions can, so to speak, de-differentiate and develop into neural-type retinal cells (275).

In lower vertebrates capable of rapid coloration changes, neural factors influence pigmentation in striking fashion. These neural mechanisms involve afferent pathways which are usually visual impulses. Blinding these animals generally causes paling of their skins. The efferent pathways influencing coloration act in part by activating endocrine organs, such as the hypophysis or the adrenals, which, in turn, produce hormones which regulate chromatophores. In part, however, these efferent pathways, particularly in creatures like the flounder, must actually exert a direct influence on the chromatophores; otherwise accurate pattern effects could not be produced.

A double antagonistic autonomic innervation of contractile chromatophores has long been claimed in lower vertebrates, with sympathetic impulses causing "contraction" and parasympathetic impulses causing "expansion" of these cells (247). Parker *et al.* (217) analyzed the dark dorsal and pale

ventral skin of the catfish, *Ameiurus*, and found 0.02–0.08 γ of acetylcholine per gram of moist skin in the dark portions and only 0.005–0.04 γ in the pale portions. This observation harmonizes well with the idea that chromatophore-expanding fibers are parasympathetic (cholinergic) in type.

In man there are numerous clinical observations and experiments that suggest neural control over melanogenesis, but conclusive proof is lacking. The transplantation experiments in vitiligo discussed on page 541 can most easily be interpreted on the basis of such control. Burn (36) has even postulated a mechanism by which the sympathetic nervous system may participate in melanogenesis. After pointing out the chemical similarity between the precursors of nor-epinephrine and melanin and calling attention to Goodall's demonstration of dopa in sheep adrenal medullae by paper chromatography, he suggests that sympathetic nerves may carry dopa to the skin, where, in turn, it can stimulate or be used for melanin formation.

Ito (127) further calls attention to clinical cases of systematized nevi, incontinentia pigmenti, nevus ophthalmo-maxillaris Ota, and Mongolian spot, where pigmentary changes are sometimes localized along cutaneous nerve distributions. He also points out that functional abnormalities of the vegetative nervous system as disclosed by

histamine and diaphoretic tests have been correlated with local pigmentary disturbances. Thus in vitiligo, hypo-esthesia (145, 251) and local autonomic reflex disturbances (164) have been reported by several observers, though denied by others (251,

109). Some others (301, 117, 94) have also reported abnormal pigmentation in association with neurologic or psychiatric disturbances. Much work still remains to be done to evaluate fully the relationship of neural factors to melanogenesis.

VIII. FACTORS INFLUENCING SKIN MELANIN CONTENT

Obvious variations in the amount of melanin in the skin and hair occur in both normal and abnormal conditions. These variations may be dependent either on variations

albino skin, all contain approximately the same numbers of dendritic melanocytes (9). In albinism, a recessively inherited anomaly found throughout the mammalian world,

TABLE 1
CLASSIFICATION OF MELANIN PIGMENTATION OF THE SKIN (132)

MELANOSIS NOT ASSOCIATED WITH AN INCREASED NUMBER OF MELANOBLASTS			MELANOSIS ASSOCIATED WITH INCREASED NUMBER OF MELANOBLASTS
EXTERNAL CAUSES	INTERNAL CAUSES		
	Conditions Primarily of General Medical Interest	Conditions Primarily of Dermatologic Interest	
Sunlight Ultraviolet rays Roentgen rays Alpha rays from thorium X Photosensitization to actinic rays Heat rays Mechanical irritation Chemical irritation Vagabonds' disease	Vitamin A deficiency* Vitamin C deficiency* Pellagra* Sprue* Anemias* Lymphomas* Gaucher's disease* Niemann-Pick's disease* Acanthosis nigricans Scleroderma Ingestion of arsenic Vagabonds' disease*† Ochronosis (alcaptonuria)*‡ Addison's disease Acromegaly* Cushing's disease* Hyperthyroidism* Pregnancy Chloasma uterinum Hemochromatosis (many cases)* Additional miscellaneous causes*	<i>Erythrose peribuccale pigmentaire</i> Postinflammatory condition Chronic dermatitis Dermatitis herpetiformis Urticaria with pigmentation Urticaria pigmentosa Xeroderma pigmentosum War melanosis (Riehl) Lentigo Additional miscellaneous causes	Ephelides (freckles) Pigmented nevus Neoplasms Melanoma Melanotic epithelioma Melanotic carcinoma Mongolian spot Blue nevus Neurofibromatosis Albright's syndrome* Leschke's syndrome* Congenital neurocutaneous syndrome of melanosis of skin and central nervous system* Skin pigment spot localized over spina bifida occulta*

* Diseases added to original classification, on the basis either of data in the literature or of their similarity to disorders already classified.
† Vagabonds' disease may have an internal as well as an external cause.
‡ Pigmentation chiefly in cartilage; skin pigmentation less constant and in corium originates from homogentisic acid (207).

in numbers of melanocytes or simply on the physiological activities of these cells in forming melanin. Normal skin of all colors, including Negro skin, vitiliginous skin, and

there seems to be a genetic lack of the enzyme tyrosinase, while in Negro skin, on the other hand, there is a genetically controlled hyperactivity of this enzymatic function.

The mechanisms by which genetic factors regulate melanin production so as to account for the many normal variations in color, shade, and patterning of melanin pigmentation throughout the animal world are largely not understood. Many of the known mechanisms regulating melanin formation have already been discussed.

The rate at which melanin is lost from the skin must also obviously influence its melanin content. Two pathways of excretion of melanin granules are known. One is up-

riod for leukoderma to become manifest after exposure to monobenzyl ether of hydroquinone can be taken as an indication of this rate.

Variations in numbers of melanocytes are also important in determining skin melanin content, especially in abnormal conditions. Becker and Obermeyer (10) proposed a classification of various melanoses on the basis of whether they were associated with increased numbers of melanocytes or not. Jeghers' extension and modification of this

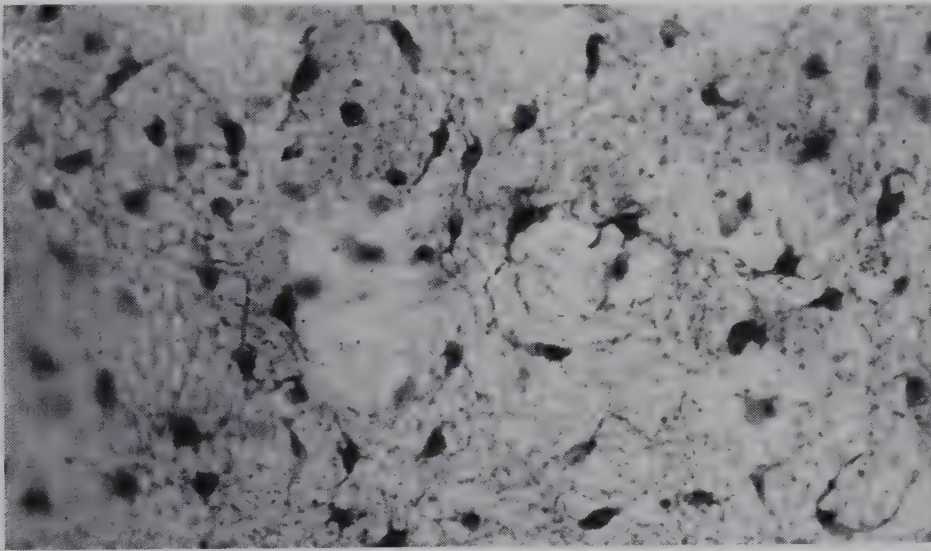


FIG. 11.—Dermal surface of separated epidermis 39 days after application of thorium X; melanocytes are identical with those seen in normal skin and contain melanin pigment in the form of nuclear caps; dopa. From Becker, Fitzpatrick, and Montgomery (9). (Reproduced by permission of Dr. Becker and the American Medical Association.)

ward through the epidermis, with ultimate loss from the surface along with the physiological shedding of the horny layer. The second is through the corium into macrophages, the lymphatic system, and ultimately perhaps even into the blood stream and from there into various organs and the urine. Loss of melanin into the corium is favored under pathologic conditions where the basal layer becomes disrupted, such as lichen planus, lupus erythematosus, and incontinentia pigmenti. On the other hand, loss of melanin from the surface is favored under conditions of increased desquamation. Normally the rate of melanin loss from the epidermis seems to be slow if the long latent pe-

classification are shown in Table 1. Whether such a sharp classification as given is applicable to all conditions listed in the table is open to question, and it is probable that combinations of both factors—physiological activity and numbers of melanocytes—can be simultaneously involved to greater or lesser extent. In the case of melanosis from thorium X, for example, Becker (9) has noted an increase in numbers as well as in activity of melanocytes (Fig. 11).

No detailed discussion of the many clinical conditions associated with abnormalities in melanin pigmentation will be attempted here. Jeghers (132) covers this subject well in his review article.

IX. SUN-TANNING

Sunlight irradiation brings about darkening of skin through several mechanisms worthy of comment. An initial darkening effect often appears a few minutes after exposure and reaches a maximum within 1 hour. This is probably a type of Meirowsky phenomenon and is brought about by the relatively long-wave ultraviolet spectrum between 300 and 420 $m\mu$, with a maximally effective wave length at 340 $m\mu$ (122). This pigment-darkening effect is believed to be a photo-oxidation of preformed relatively reduced melanin (194). It is abolished if the oxygen supply to the skin is diminished, as by blanching, with pressure applied with a quartz plate.

A second effect occurs several days after sunlight exposure and consists of an upward migration of melanin granules in the epidermis (29), probably as a result of dispersion of these granules within the dendritic melanocytes. At times as a consequence of this granule migration, the basal layer may appear relatively free of melanin granules.

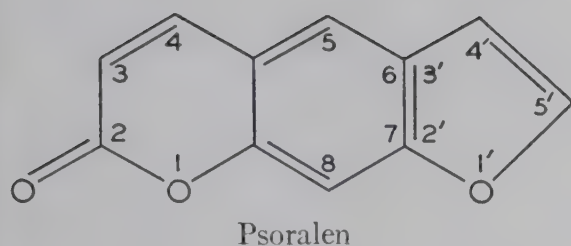
Finally, a third important and well-known factor, that of new formation of melanin, is involved in sun-tanning. According to Edwards and Duntley (62), this begins after 2 days and reaches a maximum after 19 days. After 1 month the amount of newly formed melanin begins to decline, and after 9½ months the melanin content of the skin is almost back to normal. This new formation of melanin is caused by the shorter-wave-length erythema-producing ultraviolet spectrum between 280 and 310 $m\mu$. It is of interest to point out here that many sun-screening preparations, especially those based on para-aminobenzoic acid, effectively filter the erythema wave lengths of sunlight, yet permit the passage of pigment-darkening rays above 320 $m\mu$ (250). This well explains the failure of such preparations to protect against the darkening effect of sunlight on freckles (68).

Various explanations have been suggested for the new formation of melanin which occurs after ultraviolet irradiation, and possibly all of these play a role in the process (157). These are: (1) a drop in tyrosinase-inhibitory epidermal sulfhydryl groups occurs from such radiation; (2) ultraviolet radiation catalyzes the oxidation of tyrosine to dopa, and the small amounts of dopa thus formed then catalyze the tyrosine-tyrosinase reaction; (3) the redox potential of the skin changes with irradiation, so as to favor dark melanin formation; and (4) increased skin temperature accompanying the ultraviolet erythema accelerates the tyrosine-tyrosinase reaction.

Rothman in 1923 (241) noted that with the development of sun-tanning the tyrosine level of blood serum drops considerably. The present writer was able to confirm these early observations in a few additional instances (171). It was concluded that after ultraviolet irradiations, circulating tyrosine is taken up by the "activated" epidermal melanocytes to form melanin.

Various photosensitizing substances increase the pigmentogenic effects of sunlight and, furthermore, often shift the effective wave lengths to those strongly absorbed by the particular compound. These wave lengths do not in many cases correspond with the usual sunburn spectrum. For example, the psoralen (furo-coumarin) derivatives, which are the active photosensitizers in many plants,⁹ sensitize to ultraviolet radiation of wave lengths above 320 $m\mu$.

9. These plants include citrus fruits (bergamot oil), parsnips, *Ammi majus* (xanthotoxin, ammoidin; bergapten, majudin; imperatorin, ammidin), *Imperatoria ostruthium*, *Fagara xanthoxyloides*, *Angelica archangelica*, *A. glabra*, *Seseli indicum*, *Skimmia laureola*, *Aegle marmelos*, *Ruta chalapensis*, *R. graveoleus*, *Luvanga scandens*, *Psoralea corylifolia*, and others (Aaron Bunsen Lerner, Cleveland R. Denton, and Thomas B. Fitzpatrick, Clinical and experimental studies with 8-methoxypsoralen in vitiligo, J. Invest. Dermat., 20:299-314, 1953).



The subject of photosensitization and photosensitizers is well reviewed in a monograph by Blum.¹⁰

10. H. F. Blum, Photodynamic action and diseases caused by light ("American Chemical Society monograph series") (New York: Reinhold Publishing Corp., 1941).

BIBLIOGRAPHY

1. ABDERHALDEN, E. The effect of vitamin C (ascorbic acid) upon the action of tyrosinase, *Fermentforsch.*, **14**:367-69, 1934.
2. ABT, A. F., and FARMER, C. J. Vitamin C-pharmacology and therapeutics, *J.A.M.A.*, **111**:1555-65, 1938.
3. ADACHI, B. Hautpigment beim Menschen und bei den Affen, *Ztschr. f. Morphol. u. Anthropol.*, **6**:1-131, 1913.
4. ALSBERG, C. L. On the occurrence of oxidative ferments in a melanotic tumor of the liver, *J. M. Research*, **16**:117-19, 1907-8.
5. ALTMAN, A., and MURRAY, J. F. The anemia of malignant malnutrition (infantile pellagra, kwashiorkor): protein deficiency as a possible etiological factor, *South African J. M. Sc.*, **13**:91-113, 1948.
6. ANSBACHER, S. *p*-Aminobenzoic acid, a vitamin, *Science*, **93**:164-65, 1941.
7. ARNOW, L. E. The formation of dopa by the exposure of tyrosine solutions to ultraviolet radiation, *J. Biol. Chem.*, **120**:151-53, 1937.
8. BALL, E. G., and CHEN, T. T. Studies on oxidation reduction. XX. Epinephrine and related compounds, *J. Biol. Chem.*, **102**: 691-719, 1933.
9. BECKER, S. W., JR.; FITZPATRICK, T. B.; and MONTGOMERY, H. Human melanogenesis: cytology and histology of pigment cells (melanodendrocytes), *Arch. Dermat. & Syph.*, **65**:511-23, 1952.
10. BECKER, S. W., and OBERMAYER, M. E. *Modern dermatology and syphilology*. Philadelphia: J. B. Lippincott Co., 1940.
11. BEER, R. J. S.; McGRATH, L.; ROBERTSON, A.; and WOODIER, A. B. The chemistry of melanins. II. The synthesis of 5,6-dihydroxyindole-2-carboxylic acid and related compounds, *J. Chem. Soc.*, pp. 2061-66, 1949.
12. BELL, R., and LORINCZ, A. L. Unpublished experiments.
13. BERGENSTAL, D. M. Personal communication.
14. BERNHEIM, F., and BERNHEIM, M. L. C. The action of phenyl thiocarbamide on tyrosinase, *J. Biol. Chem.*, **145**:213-17, 1942.
15. BERNSTEIN, E. T., and SACH, P. M. Pigmentation disturbance following exposure to monobenzyl ether of hydroquinone, *Arch. Dermat. & Syph.*, **59**:542-48, 1949.
16. BERTRAND, G. Sur une nouvelle oxidase, ou ferment soluble oxydant, d'origine végétale, *Compt. rend. Acad. d. sc.*, **122**:1215-17, 1896.
17. BILLINGHAM, R. E. Dendritic cells, *J. Anat.*, **82**:93-109, 1948.
18. ———. Dendritic cells in pigmented human skin, *ibid.*, **83**:109-15, 1949.
19. BILLINGHAM, R. E., and MEDAWAR, P. B. Pigment spread and cell heredity in guinea pigs' skin, *Heredity*, **2**:29-47, 1948. See also: A study of the branched cells of the mammalian epidermis with special reference to the fate of their division products, *Phil. Tr. Roy. Soc. London, s.B.*, **237**:151-71, 1953.
20. ———. Pigment spread in mammalian skin. Serial propagation and immunity reactions, *Heredity*, **4**:141-64, 1950.
21. BLACKBERG, S. N., and WANGER, J. O. Melanuria, *J.A.M.A.*, **100**:334-36, 1933.
22. BLOCH, B. Chemische Untersuchungen über das spezifische pigmentbildende Ferment der Haut, die Dopaoxydase, *Ztschr. f. physiol. Chem.*, **98**:226-54, 1916.
23. ———. Das Pigment. In: JADASSOHN, *Handb. d. Haut- u. Geschlechtskr.*, **1/1**: 434-51. Berlin: J. Springer, 1927.
24. ———. Quoted by Bloch (23, n. 1).
25. ———. Problem of pigment formation, *Am. J. M. Sc.*, **177**:609-18, 1929.
26. BLOCH, B., and LÖFFLER, W. Untersuchungen über die Bronzefärbung der Haut bei der Addison'schen Krankheit, *Deutsches Arch. f. klin. Med.*, **121**:262-91, 1917.

27. BLOCH, B., and SCHAAF, F. Pigmentstudien, *Biochem. Ztschr.*, **162**:181-206, 1925.
28. BLOCH, B., and SCHRAFL, A. Experimentelle Untersuchungen über den Einfluss des Ovarialhormons auf die Pigmentbildung, *Arch. f. Dermat. u. Syph.*, **165**:268-93, 1932.
29. BLUM, H. F. The physiological effects of sunlight on man, *Physiol. Rev.*, **25**:483-529, 1945.
30. BOEHM, F. Beitrag zur Kenntnis der Thormählenschen Reaktion, *Ztschr. f. physiol. Chem.*, **258**:108-16, 1939.
31. BOURQUELOT, E., and BERTRAND, G. Le bluissement et le noircissement des champignons, *Compt. rend. Soc. de biol.*, **47**:582-84, 1895.
32. BOWNESS, J. M.; MORTON, R. A.; SHAKIR, M. H.; and STUBBS, A. L. Distribution of copper and zinc in mammalian eyes. Occurrences of metals in melanin fractions from eye tissues, *Biochem. J.*, **51**:521-29, 1952.
33. BRUNSTING, L. A., and SHEARD, C. Color of skin as analyzed by spectrophotometric methods. II. Role of pigmentation, *J. Clin. Investigation*, **7**:575-92, 1929.
34. BU'LOCK, J. D.; HARLEY-MASON, J.; and MASON, H. S. Hallachrome and dopachrome, *Biochem. J.*, **47**:xxxii, 1950.
35. BUNAK, W. W. Über die Variationen des Pigments und ihre Bedeutung für die Variation der Haarfarbe, *Chem. Zentralbl.*, **1**:3008, 1939.
36. BURN, J. H. Pigmentation of the skin, *Brit. J. Dermat.*, **63**:431-40, 1951.
37. BURTON, H. The constitution of melanin, *Chem. & Indust.*, p. 313, 1948.
38. BURTON, H.; DUFFIELD, J. A., and PRAILL, P. F. G. The synthesis of derivatives of 5,6-dihydroxyindole. II. 5,6-dihydroxyindole and related compounds, *J. Chem. Soc.*, pp. 1062-65, 1950.
39. BUTCHER, E. D. Effects of adrenalectomy on pigmentation of hair in rats fed a deficient diet, *Proc. Soc. Exper. Biol. & Med.*, **60**:396-97, 1945.
40. CHARLES, D. R. Studies on spotting patterns. IV. Pattern variation and its developmental significance, *Genetics*, **23**:523-47, 1938.
41. CHASE, H. B. Greying of hair. I. Effects produced by single doses of X-rays on mice, *J. Morphol.*, **84**:57-80, 1949.
42. CHASE, H. B.; RAUCH, R.; and SMITH, V. W. Critical stages of hair development and pigmentation in the mouse, *Physiol. Zoöl.*, **24**:1-8, 1951.
43. CLEMO, G. R., and DUXBURY, F. K. Formation of tyrosine melanin, *J. Chem. Soc.*, pp. 1795-1800, 1950.
44. COMAR, C. L.; SINGER, L.; and DAVIS, G. K. Molybdenum metabolism and interrelationships with copper and phosphorus, *J. Biol. Chem.*, **180**:913-22, 1949.
45. COMÉL, M. Modificazioni delle alterazioni cutanee della vitiligo e della sclerodermia in zona trapianto cutaneo, *Dermatologica*, **96**:366-72, 1948. See also SPENCER, G. A., and TOLMACH, J. A. Exchange grafts in vitiligo, *J. Invest. Dermat.*, **19**:1-5, 1952.
46. CORNBLEET, T. Vitamin C and pigment, *Arch. Dermat. & Syph.*, **35**:471-79, 1937.
47. COTTER, L. Pentachlorinated naphthalenes in industry, *J.A.M.A.*, **125**:273-74, 1944.
48. DAVIS, M. E.; BOYNTON, M. W.; FERGUSON, J. H., and ROTHMAN, S. Studies on pigmentation of endocrine origin, *J. Clin. Endocrinol.*, **5**:138-46, 1945.
49. DAWES, B. The melanin content of the skin of *Rana temporaria* under normal conditions and after prolonged light- and dark-adaptation; a photometric study, *J. Exper. Biol.*, **18**:26-49, 1941.
50. ———. Pigmentary changes and the background response in Amphibia, *Nature*, **147**:806-7, 1941.
51. DAWSON, J. W. The melanomata; their morphology and histogenesis; a study of cell origin and transformations with a critical discussion on aspects of tumor growth and a clinical review, *Edinburgh M. J.*, **32**:501-732, 1925.
52. DE COULON, A. Étude du pigment retiré d'un mélanome de cheval, *Compt. rend. Soc. de biol.*, **83**:1451-53, 1920.
53. DENTON, C. R.; LERNER, A. B.; and FITZPATRICK, T. B. Inhibition of melanin formation by chemical agents, *J. Invest. Dermat.*, **18**:119-35, 1952. See also LERNER, A. B., and FITZPATRICK, T. B., Treatment of melanin hyperpigmentation, *J.A.M.A.*, **152**:577-82, 1953; and FORMAN, L., A note on the depigmentary properties of monobenzylether of hydroquinone, *Brit. J. Dermat.*, **65**:406-9, 1953.
54. DIECKE, S. H. Pigmentation and hair growth in black rats, as modified by the

- chronic administration of thiourea, phenylthiourea and α -naphthyl thiourea, *Endocrinology*, **40**:123-36, 1947.
55. DIMICK, M. K., and LEPP, A. Relation of pantothenic acid to filtrate fraction of vitamin B complex, *J. Nutrition*, **20**:413-26, 1940.
56. DIXON, H. A. Melanotic sarcoma with extreme melanosis; report of case, *Arch. Dermat. & Syph.*, **38**:574-82, 1938.
57. DOBROVOLSKAIA-ZAVADSKAIA, N., and RODZEVITCH, M. Panachure artificielle chez des souris irradiées, *Compt. rend. Soc. de biol.*, **135**:1303-5, 1941.
58. DUBOIS, K. P., and ERWAY, W. F. Studies on the mechanism of action of thiourea and related compounds. II. Inhibition of oxidative enzymes and oxidations catalyzed by copper, *J. Biol. Chem.*, **165**:711-21, 1946.
59. DURHAM, F. M. On the presence of tyrosinases in the skins of some pigmented vertebrates, *Proc. Roy. Soc. London, s.B.*, **74**:310-13, 1904-5.
60. DU SHANE, G. P. The development of pigment cells in vertebrates. *In*: MINER, R. W. (ed), *The biology of melanomas*, pp. 1-14. ("Special publications of the New York Academy of Sciences," Vol. 4.) New York: The Academy, 1948.
61. DUTCHER, T. F., and ROTHMAN, S. Iron, copper and ash content of human hair of different colors, *J. Invest. Dermat.*, **17**:65-68, 1951.
62. EDWARDS, E. A., and DUNTLEY, S. Q. Pigments and color of living human hair, *Am. J. Anat.*, **65**:1-33, 1939.
63. EDWARDS, E. A.; HAMILTON, J. B.; DUNTLEY, S. Q.; and HUBERT, G. Cutaneous vascular and pigmentary changes in castrate and eunuchoid men, *Endocrinology*, **28**:119-28, 1941.
64. EMERSON, G. A., and EVANS, H. M. Growth and graying of rats with total "filtrate factor" and with pantothenic acid, *Proc. Soc. Exper. Biol. & Med.*, **46**:655-58, 1941.
65. EMERSON, G. A., and KERESZTESY, J. C. Biotin deficiency in rats, *Proc. Soc. Exper. Biol. & Med.*, **51**:358-61, 1942.
66. EPPINGER, H. Über Melanurie, *Biochem. Ztschr.*, **28**:181-92, 1910.
67. EVANS, W. C., and RAPER, H. S. The accumulation of *l*-3,4-dihydroxyphenylalanine in the tyrosine-tyrosinase reaction, *Biochem. J.*, **31**:2162-70, 1937.
68. FELSHER, Z.; RUBIN, L.; and ROTHMAN, S. Studies on the darkening of freckles, *Dermatologica*, **94**:280-85, 1947.
69. FERGUSON, W. S.; LEWIS, A. H.; and WATSON, S. J. The cause and cure of teartness, *J. Agr. Sc.*, **33**:44-51, 1943.
70. FIGGE, F. H. J. Factors regulating the formation and physical and chemical properties of melanin. *In*: MINER, R. W. (ed.), *The biology of melanomas*, pp. 405-21. ("Special publications of the New York Academy of Sciences," Vol. 4.) New York: The Academy, 1948.
71. FIGGE, F. H. J., and ALLEN, E. Release of glutathione inhibition by estrone, *Endocrinology*, **29**:262-66, 1941.
72. FISHER, A. A. Treatment of chloasma with monobenzyl ether of hydroquinone, *Arch. Dermat. & Syph.*, **64**:645-46, 1951.
73. FITZPATRICK, T. B. Human melanogenesis, *Arch. Dermat. & Syph.*, **65**:379-91, 1952.
74. FITZPATRICK, T. B.; BECKER, S. W., JR.; LERNER, A. B.; and MONTGOMERY, H. Tyrosinase in human skin: demonstration of its presence and its role in human melanin formation, *Science*, **112**:223-25, 1950.
75. FLESCH, P. The role of copper in mammalian pigmentation, *Proc. Soc. Exper. Biol. & Med.*, **70**:79-83, 1949.
76. ———. Inhibitory action of extracts of mammalian skin on pigment formation, *ibid.*, pp. 136-40.
77. FLESCH, P., and ROTHMAN, S. Isolation of iron pigment from human red hair, *J. Invest. Dermat.*, **6**:257-70, 1945.
78. FORBES, T. R. Sex hormones and hair changes in rats, *Endocrinology*, **30**:465-68, 1942.
79. FOSTER, M. Enzymatic studies of pigment-forming abilities in mouse skin, *J. Exper. Zool.*, **117**:211-46, 1951.
80. FOSTVEDT, G. A. Effect of high melanophore hormone fractions on tyrosine and dopa oxidation, *Endocrinology*, **27**:100-109, 1940.
81. FRIEDEN, E. H., and BOZER, J. M. Effect of administration of intermedin upon melanin content of skin of *Rana pipiens*, *Proc. Soc. Exper. Biol. & Med.*, **77**:35-37, 1951.
82. FRITZ, J. C.; HOOPER, J. H.; HALPIN, J. L.; and MOORE, H. P. Failure of feather pigmentation in bronze poult due to ly-

- sine deficiency, *J. Nutrition*, **31**:387-96, 1946.
83. FROST, D. V. The relation of nutritional deficiencies to graying, *Physiol. Rev.*, **28**:368-82, 1948.
84. FROST, D. V., and DANN, F. P. Unidentified factor(s) in yeast and liver essential to the cure of achromotrichia in dogs on synthetic diets, *J. Nutrition*, **27**:355-62, 1944.
85. FROST, D. V.; DANN, F. P.; and MCINTIRE, F. C. Adequacy of the known synthetic vitamins for normal feathering and pigmentation in chicks, *Proc. Soc. Exper. Biol. & Med.*, **61**:65-69, 1946.
86. FROST, D. V.; DANN, F. P.; and MOORE, R. C. Effect of pantothenic acid alone and in natural products on nutritional achromotrichia in rats, *Proc. Soc. Exper. Biol. & Med.*, **46**:507-11, 1941.
87. FÜRTH, O. VON, and SCHNEIDER, H. Ueber tierische Tyrosinasen und ihre Bedeutung zur Pigmentbildung, *Beitr. z. chem. Physiol. u. Path.*, **1**:229-42, 1901.
88. GEILING, E. M. K., and OLDHAM, F. K. The neurohypophysis, *J.A.M.A.*, **116**:302-6, 1941.
89. GESCHWIND, I. I.; REINHARDT, W. O.; and LI, C. H. Observations on the connexion between intermedin and adrenocorticotrophic hormone, *Nature*, **169**:1061, 1952.
90. GESSARD, C. Sur la formation du pigment mélanique dans les tumeurs du cheval, *Compt. rend. Acad. d. Sc.*, **136**:1086-88, 1903.
91. GILLMAN, T., and GILLMAN, J. Powdered stomach in treatment of fatty liver and other manifestations of infantile pellagra, *Arch. Int. Med.*, **76**:63-74, 1945.
92. ———. Hepatic damage in infantile pellagra and its response to vitamin, liver, and dried stomach therapy as determined by repeated liver biopsies, *J.A.M.A.*, **129**:12-19, 1945.
93. GINSBERG, B. The effects of the major genes controlling coat color in the guinea pig on the dopa oxidase activity of skin extracts, *Genetics*, **29**:176-98, 1944.
94. GOLDSMITH, W. N. Recent advances in dermatology. Philadelphia: P. Blakiston's Son & Co., 1936.
95. GOLDZIEHER, J. A.; ROBERTS, J. S.; RAWLS, W. B.; and GOLDZIEHER, M. A. Chemical analysis of the intact skin by reflectance spectrophotometry, *Arch. Dermat. & Syph.*, **64**:533-48, 1951.
96. GORDON, M. (ed.). Pigment cell growth, preface. New York: Academic Press, Inc., 1953.
97. GORTER, F. J. Depigmentation, a new dietary deficiency disease, cured by copper, *Nature*, **136**:185, 1935.
98. GRAND, C. G., and CAMERON, G. Tissue culture studies of pigmented melanomas: fish, mouse and human. In: MINER, R. W. (ed.), *The biology of melanomas*, pp. 171-76. ("Special publications of the New York Academy of Sciences," Vol. 4.) New York: The Academy, 1948.
99. GRAUBARD, M., and PINCUS, G. Steroid metabolism: estrogens and phenolases, *Endocrinology*, **30**:265-69, 1942.
100. GREENSTEIN, J. P.; TURNER, F. C.; and JENRETTE, W. V. Chemical studies on the components of normal and neoplastic tissues. V. The melanin-containing pseudoglobulin of the malignant melanoma of mice, *J. Nat. Cancer Inst.*, **1**:377-85, 1940.
101. GROODY, T. C., and GROODY, M. E. Feather depigmentation and pantothenic acid deficiency in chicks, *Science*, **95**:655-56, 1942.
102. GROSS, P.; RUNNE, E.; and WILSON, J. W. Studies on the effect of thallium poisoning of the rat. The influence of cystine and methionine on alopecia and survival period, *J. Invest. Dermat.*, **10**:119-34, 1948.
103. GRUNDEBERG, T., and SCHADE, H. Zur Frage der Beeinflussung der Dopareaktion durch Vitamin C (Ascorbinsäure), *Klin. Wchnschr.*, **13**:1353-54, 1934.
104. GRUPPER, C.; PLAS, G.; and BAUDIN, P. Cinq cas de mélanose de Riehl guéris ou améliorés par le corps H-365 (para oxypropionophénone). Conceptions actuelles de la mélanogenèse, *Bull. Soc. franç. de dermat. et syph.*, **57**:346-51, 1950.
105. GYÖRGY, P., and POLING, C. E. Further experiments on nutritional achromotrichia in rats and mice, *Proc. Soc. Exper. Biol. & Med.*, **45**:773-76, 1940.
106. ———. Pantothenic acid and nutritional achromotrichia in rats, *Science*, **92**:202-3, 1940.
107. GYÖRGY, P.; POLING, C. E.; and SUBBAROW, Y. Observations on the factor curative of nutritional achromotrichia, *J. Biol. Chem.*, **132**:789-90, 1940.
108. HABERMAN, R. Paratypische Pigmentanomalien. In: JADASSOHN, *Handb. d.*

- Haut- u. Geschlechtskr., 4/2:796-970. Berlin: J. Springer, 1933.
109. ———. Vitiligo. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., 4/2:896-930. Berlin: J. Springer, 1933.
 110. HADEN, R. L., and ORR, T. G. Melanuria in the absence of melanotic tumor, Bull. Johns Hopkins Hosp., 35:58-63, 1924.
 111. HALL, T. C.; MCCracken, B. H.; and THORN, G. W. Skin pigmentation in relation to adrenal cortical function, J. Clin. Endocrinol. & Metabol., 13:243-57, 1953.
 112. HAMILTON, H. L. Influence of sex hormones and desoxycorticosterone on melanophore differentiation in birds, Proc. Soc. Exper. Biol. & Med., 45:571-73, 1940.
 113. HAMILTON, J. B. Significance of sex hormones in tanning of the skin of women, Proc. Soc. Exper. Biol. & Med., 40:502-3, 1939.
 114. ———. Influence of the endocrine status upon the pigmentation in man and in mammals. In: MINER, R. W. (ed.), The biology of melanomas, pp. 341-57. ("Special publications of the New York Academy of Sciences," Vol. 4.) New York: The Academy, 1948.
 115. HAMILTON, J. B., and HUBERT, G. Photographic nature of tanning of the human skin as shown by studies of male hormone therapy, Science, 88:481, 1938.
 116. HANNA, B. L. Production of pigments similar to those from hair from amino acid and keratin interaction, Proc. Soc. Exper. Biol. & Med., 80:285-87, 1952.
 117. HARRIS, A. Skin pigmentation with dementia, Lancet, 2:125, 1942.
 118. HARRIS, SEALE, and HARRIS, SEALE, JR. Clinical pellagra. St. Louis: C. V. Mosby Co., 1941.
 119. HARTWELL, G. A. Note on the colour changes in rat's fur produced by alterations in diet, Biochem. J., 17:547-48, 1923.
 120. HAXTHAUSEN, H. Studies on the pathogenesis of morphea, vitiligo and acrodermatitis atrophicans by means of transplantation experiments, Acta dermat.-venereol., 27:352-68, 1947.
 121. HELLERSTEIN, H. K. Personal communication cited by LERNER and FITZPATRICK (157).
 122. HENSCHKE, V., and SCHULZE, R. Über Pigmentierung durch langwelliges Ultraviolett, Strahlentherapie, 64:14-42, 1939.
 123. HÖRSTADIUS, S. The neural crest. London: Oxford University Press, 1950.
 124. HOGEBOM, G. H., and ADAMS, M. H. Mammalian tyrosinase and dopa oxidase, J. Biol. Chem., 145:273-79, 1942.
 125. HOUSSAY, B. A., and UNGER, I. Action de l'hypophyse sur la coloration des batraciens, Compt. rend. Soc. de biol., 91:318-20, 1924.
 126. HUNDLEY, J. M., and ING, R. B. Effect of panthothenic acid deficiency on skin copper, Federation Proc., 10:385, 1951.
 127. ITO, M. Studies on melanin, Tohoku J. Exper. Med., 55 (suppl. 1): 101-2, 1952.
 128. JACOBSON, V. C. Melanin; review of chemical aspects of melanin problem, Arch. Path., 17:391-403, 1934.
 129. JADASSOHN, W.; UEHLINGER, E.; and MARGOT, A. Nipple test; studies in local and systemic effects on topical application of various sex hormones, J. Invest. Dermat., 1:31-43, 1938.
 130. JANKOWSKY, W. Beitrag zur der Haarpigmente, Ztschr. f. Rassenphysiol., 5:1-48, 1932.
 131. JAQUES, R. Action inhibitrice du thiouracil sur la mélanogenèse de la queue d'axolotl en régénération, Experientia, 6:148-49, 1950.
 132. JEGHERS, H. Pigmentation of the skin, New England J. Med., 231:88-100, 122-36, 181-89, 1944.
 133. JERVIS, G. A. Studies on phenylpyruvic oligophrenia. The position of the metabolic error, J. Biol. Chem., 169:651-56, 1947.
 134. JESIONEK, A. Heliotherapie und Pigment, Ztschr. f. Tuberk., 24:401-14, 1915.
 135. ———. Biologie der gesunden und kranken Haut. Leipzig: F. C. W. Vogel, 1916.
 136. JODAR, E. O. Tanning from UVL irradiation brought out by blood transfusion, Am. J. Dis. Child., 58:1047-49, 1939.
 137. JOHNSON, S., and HÖGBERG, B. Observations on the connection between intermedin and adrenocorticotrophic hormone, Nature, 169:286, 1952.
 138. JOY, H. H. Uveopigmentary sensitization, Am. J. Ophth., 31:1581-88, 1948.
 139. KASTLE, J. H. The oxidases and other oxygen catalysts concerned in biological oxidations. (U.S. Hygienic Laboratory Bull. 59.) Washington: Government Printing Office, 1910.
 140. KAUSCHE, G. A., and HAHN, F. Über die

- Einwirkung von Tyrosinase auf Proteine, Ztschr. f. Naturforsch., **4**:223-26, 1949.
141. KEIL, H. L., and NELSON, V. E. The role of copper in hemoglobin regeneration and in reproduction, J. Biol. Chem., **93**:49-57, 1931.
 142. KERTESZ, D. Tyrosinase and polyphenol-oxidase; the role of metallic ions in melanogenesis, Nature, **168**:697, 1951.
 143. KEYS, A. Caloric undernutrition and starvation with notes on protein deficiency, J.A.M.A., **138**:500-511, 1948.
 144. KÖNIGSBAUER, H., and THALLER, G. Experimentell erzeugte Poliosis circumscripta und Papillombildung durch Stickstofflost bei der schwarzen Maus, Arch. f. Dermat. u. Syph., **190**:493-99, 1950.
 145. KÖNIGSTEIN, H. Sensibilitätsstörung bei Vitiligo, Wien. klin. Wchnschr., **23**:1745-48, 1910.
 146. KRÜGER, L. Spektralanalytische Untersuchungen von Haarfarben, Wissensch. Arch. f. Landwirtsch., **1**:52-122, 1929.
 147. KUBOWITZ, F. Über die chemische Zusammensetzung der Kartoffeloxydase, Biochem. Ztschr., **292**:221-29, 1937.
 148. ———. Spaltung und Resynthese der Polyphenoloxydase und des Hämocyanins, *ibid.*, **299**:32-57, 1938.
 149. KUPPERMAN, H. S. Hormone control of a dimorphic pigmentation area in the golden hamster (*Cricetus auratus*), Anat. Rec., **88**:442, 1944.
 150. LATARJET, R. La physiologie normale du pigment mélanique cutané chez l'homme, Biol. méd. Paris, **28**:65-104, 1938.
 151. LEA, A. J. Influence of sodium chloride on formation of melanin, Nature, **155**:428-29, 1945.
 152. ———. A neutral solvent for melanin, *ibid.*, **156**:478, 1945.
 153. ———. Effect of hydroquinone monobenzyl ether on melanin formation in vitro, *ibid.*, **167**:906, 1951.
 154. LEGG, J. W. Multiple melanotic sarcomata beginning in the choroid, followed by pigmentation of the skin of the face and hands, Tr. Path. Soc. London, **35**:367-72, 1884.
 155. LÉOBARDY, J. DE, and LABESSE, A. Du traitement de la maladie d'Addison par la cysteine, Presse méd., **42**:599-600, 1934.
 156. LERNER, A. B. Effect of ions on melanin formation, J. Invest. Dermat., **18**:47-52, 1952.
 157. LERNER, A. B., and FITZPATRICK, T. B. Biochemistry of melanin formation, Physiol. Rev., **30**:91-126, 1950.
 158. ———. Personal communication.
 159. LERNER, A. B.; FITZPATRICK, T. B.; CALKINS, E.; and SUMMERSON, W. H. Enzymatic oxidation of tyrosine and dihydroxyphenylalanine by melanoma extracts, Federation Proc., **7**:176, 1948.
 160. ———. Mammalian tyrosinase: preparation and properties, J. Biol. Chem., **178**:185-95, 1949.
 161. ———. Mammalian tyrosinase: the relationship of copper to enzymatic activity, *ibid.*, **187**:793-802, 1950.
 162. ———. Mammalian tyrosinase: action on substances structurally related to tyrosine, *ibid.*, **191**:799-806, 1951.
 163. LERNER, A. B.; FITZPATRICK, T. B.; and SUMMERSON, W. H. Mammalian tyrosinase: action on compounds structurally related to tyrosine and dihydroxyphenylalanine, Federation Proc., **8**:218, 1949.
 164. LÉVY-FRANCKEL, A., and JUSTER, E. Vitiligo avec troubles nerveux sensitifs et sympathiques; l'origine sympathique du vitiligo, Bull. Soc. franç. de dermat. et syph., **28**:338-42, 1922, and **32**:342-45, 1925.
 165. LEWIN, M. L., and PECK, S. M. Pigment studies in skin grafts on experimental animals, J. Invest. Dermat., **4**:483-504, 1941.
 166. LIEBOW, A. A.; WARREN, S.; and DE COURSEY, E. Pathology of atomic bomb casualties, Am. J. Path., **25**:853-1027, 1949.
 167. LILLIE, R. J., and BRIGGS, G. M. Studies on folic acid in the prevention of abnormal feather pigmentation, Poultry Sc., **26**:475-77, 1947.
 168. LINNELL, L., and RAPER, H. S. The chromogen of melanuria, Biochem. J., **29**:76-85, 1935.
 169. LOCKHART, R. J. J., and LOEWENTHAL, J. A. Occupational leukoderma. A report of six cases observed in a South African factory, South African M. J., **23**:867-68, 1949.
 170. LORINCZ, A. L. Studies on the inhibition of melanin formation, J. Invest. Dermat., **15**:425-31, 1950.
 171. ———. Unpublished experiments.
 172. LUNDE, G., and KRINGSTAD, H. Über Veränderung des Pelzes von Ratten durch Mangel an gewissen Faktoren des Vitamin

- B-Komplexes, Ztschr. f. physiol. Chem., **257**:201-16, 1939.
173. ———. Anti-grey hair vitamin, new factor in vitamin B complex, J. Nutrition, **19**:321-32, 1940.
 174. LUSTIG, B.; GOLDFARB, A. R.; and GERSTL, B. The effect of vitamins and sex hormones on dietary achromotrichia in mice, Am. J. Physiol., **141**:259-61, 1944.
 175. LUTZ, R. E. Normal occurrence of zinc in biologic materials: a review of the literature and a study of the distribution of zinc in the rat, cat and man, J. Indust. Hyg., **8**:177-207, 1926.
 176. LYNN, W. G., and DE MARIE, SR. ALFRED. Effect of thiouracil upon the pigmentation in the tadpole, Science, **104**:31, 1946.
 177. MCGINNIS, J.; NORRIS, L. C.; and HEUSER, G. F. An unidentified nutritional factor required by the chick for feather pigmentation, J. Biol. Chem., **145**:341-42, 1942.
 178. MALLETT, M. F., and DAWSON, C. R. On the nature of highly purified mushroom tyrosinase preparations, Arch. Biochem., **23**:29-44, 1949.
 179. MARKERT, C. L. The effect of thyroxin and anti-thyroid compounds on the synthesis of pigment granules in chick melanoblasts, Physiol. Zoöl., **21**:309-27, 1948.
 180. MARTIN, G. J., and ANSBACHER, S. Confirmatory evidence of the chromotrichial activity of *p*-aminobenzoic acid, J. Biol. Chem., **138**:441, 1941.
 181. MARTIN, G. J.; WISANSKY, W. A.; and ANSBACHER, S. Para-aminobenzoic acid and dopa reaction, Proc. Soc. Exper. Biol. & Med., **47**:26-28, 1941.
 182. MASON, H. S. A classification of melanins. In: MINER, R. W. (ed.), The biology of melanomas, pp. 399-404. ("Special publications of the New York Academy of Sciences," Vol. 4.) New York: The Academy, 1948.
 183. ———. The chemistry of melanin. III. Mechanism of the oxidation of dihydroxyphenylalanine by tyrosinase, J. Biol. Chem., **172**:83-99, 1948.
 184. ———. The structure of melanins. In: GORDON, M. (ed.), Pigment cell growth, pp. 277-301. New York: Academic Press, Inc., 1953.
 185. MASON, H. S.; KAHLER, H.; MACCARDLE, R. C.; and DALTON, A. J. Chemistry of melanin. IV. Electron micrography of natural melanins, Proc. Soc. Exper. Biol. & Med., **66**:421-31, 1947.
 186. MASON, H. S., and LADA, A. Structure of a catechol and a 3,4-dihydroxyphenylalanine melanin, Federation Proc., **11**:254, 1952.
 187. MASON, H. S., and WRIGHT, C. I. The chemistry of melanin. V. Oxidation of dihydroxyphenylalanine by tyrosinase, J. Biol. Chem., **180**:235-46, 1949.
 188. MASSON, P. Pigment cells in man. In: MINER, R. W. (ed.), The biology of melanomas, pp. 15-51. ("Special publications of the New York Academy of Sciences," Vol. 4.) New York: The Academy, 1948.
 189. MAZZA, F. P., and STOLFI, G. Ricerche sul pigmento *Halla parthenopaea* Costa, Arch. di sc. biol., **16**:183-97, 1931.
 190. MEIROWSKY, E. Über Pigmentbildung in vom Körper losgelöster Haut, Frankfurter Ztschr. f. Path., **2**:438-48, 1909.
 191. ———. Idiotypische Pigmentanomalien. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **4**:2:588-795. Berlin: J. Springer, 1933.
 192. ———. A critical review of pigment research in the last hundred years, Brit. J. Dermat., **52**:205-17, 1940.
 193. MEIROWSKY, E.; FREEMAN, L. W.; and FISCHER, R. B. Observations on the structure, derivation and nature of melanin, Zoologica, **35**:29-30, 1950.
 194. MIESCHER, G., and MINDER, H. Untersuchungen über die durch langwelliges Ultraviolett hervorgerufene Pigmentdunkelung, Strahlentherapie, **66**:6-23, 1939.
 195. MOHAMMAD, A.; EMERSON, O. H.; and EVANS, H. M. Properties of the filtrate factor of the vitamin B₂ complex, with evidence for its multiple nature, J. Biol. Chem., **133**:17-28, 1940.
 196. MONCORPS, C. Studien zur Genese des normalen Oberhautpigmentes, Arch. f. Dermat. u. Syph., **148**:1-14, 1924. See also THANNHAUSER and MONCORPS, Discussion of MERTENS, V. E., Pigmentveränderungen an einem Melanoschimmel, Arch. f. Dermat. u. Syph., **145**:201-3, 1923.
 197. MORETTI, G. F. Effets de l'implantation d'ultrafiltres contenant de l'hormone mélanophorotrope sous la peau des Mammifères, Compt. rend. Soc. de biol., **141**:1060, 1947.
 198. MORGAN, A. F.; COOK, B. B.; and DAVISON, H. G. Vitamin B₂ deficiencies as af-

- fectured by dietary carbohydrate, *J. Nutrition*, **15**:27-43, 1938.
199. MORGAN, A. F., and SIMMS, H. D. Greying of fur and other disturbances due to a vitamin deficiency, *J. Nutrition*, **19**:233-50, 1940.
 200. ———. Anti-grey hair deficiency in the silver fox, *ibid.*, **20**:627-35, 1940.
 201. MORTON, A. A., and SLAUNWHITE, W. R., JR. Polymerization of 6-hydroxyindole and its relation to the formation of melanin, *J. Biol. Chem.*, **179**:259-70, 1949.
 202. MU, J. W.; FRAZIER, C. N.; and PILLAT, A. Melanin pigment of the skin and conjunctiva in avitaminosis A in man, *Chinese J. Physiol.*, **11**:247-52, 1937.
 203. MUIR, W. R. The teart (molybdenum-bearing) pastures of Somerset, *Vet. J.*, **97**:387-400, 1941.
 204. MUSSIO-FOURNIER, J. C.; CERVINO, J. M.; and CONTI, O. Treatment of vitiligo by local injections of melanophore hormone, *Endocrinology*, **28**:513-15, 1941.
 205. NEALON, D. F. Ammoniated mercury and the skin, *Drug & Cosmet. Indust.*, **52**:159-62, 1943.
 206. NELSON, J. M., and DAWSON, C. R. Tyrosinase. *In*: *Advances in enzymology*, **4**:99-152. New York: Interscience Publishers, Inc., 1944.
 207. NEUBERGER, A.; RIMMINGTON, C.; and WILSON, J. M. G. Studies on alcaptonuria, *Biochem. J.*, **41**:438-49, 1947.
 208. NICHOLLS, L. Grey hair in ill-nourished children, *Lancet*, **2**:201, 1946.
 209. NICKERSON, M. Relation between black and red melanin pigment in feathers, *Physiol. Zoöl.*, **19**:66-77, 1946.
 210. NIELSON, E.; OLESON, J. J.; and ELVEHJEM, C. A. Fractionation of the factor preventing nutritional achromotrichia, *J. Biol. Chem.*, **133**:637-38, 1940.
 211. NOBLE, G., and WURM, M. The effect of testosterone propionate on the black-crowned night heron, *Endocrinology*, **26**:837-50, 1940.
 212. ODEL, H. M.; MONTGOMERY, H.; and HORTON, B. T. Diffuse melanosis secondary to malignant melanoma: report of case, *Proc. Staff Meet. Mayo Clin.*, **12**:742-47, 1937.
 213. OETTEL, H. Die Hydrochinonvergiftung, *Arch. f. exper. Path. u. Pharmakol.*, **183**:319-62, 1936.
 214. OLIVER, E. A.; SCHWARTZ, L.; and WARREN, L. H. Occupational leukoderma, *Arch. Dermat. & Syph.*, **42**:993-1014, 1940.
 215. ONSLOW, H. A contribution to our knowledge of the chemistry of coat-colour in animals and of dominant and recessive whiteness, *Proc. Roy. Soc. London, s.B.*, **89**:36-58, 1917.
 216. ORMSBY, O. S., and MONTGOMERY, H. Diseases of the skin. Philadelphia: Lea & Febiger, 1937.
 217. PARKER, G. H.; WELSH, J. H.; and HYDE, J. E. The amounts of acetylcholine in the dark skin and in the pale skin of the catfish, *Proc. Nat. Acad. Sc.*, **31**:1-8, 1945.
 218. PASCHKIS, K. E.; CANTAROW, A.; HART, W. M.; and RAKOFF, A. E. Inhibitory action of thiouracil, thiocarbamide and other compounds on melanin formation by tyrosinase, *Proc. Soc. Exper. Biol. & Med.*, **57**:37-38, 1944.
 219. PAULAI, R.; GRACIANSKY, P. DE; and RAOUL, Y. Biochemie des mélanoses; augmentation de la teneur en cuivre du sang dans la mélanose de Riehl, *Bull. Soc. chim. biol.*, **27**:80-84, 1945.
 220. PAVCEK, P. L., and BAUM, H. M. Relation of cystine to achromotrichia, *Proc. Soc. Exper. Biol. & Med.*, **47**:271-72, 1941.
 221. PECK, S. M. Pigment (melanin) studies of the human skin after application of thorium X, *Arch. Dermat. & Syph.*, **21**:916-56, 1930.
 222. ———. The pigment melanin of the skin and hair, *J. Soc. Cosmet. Chemists*, **1**:33-45, 1947.
 223. PECK, S. M., and SOBOTKA, K. Effect of monobenzyl hydroquinone on oxidase systems in vivo and in vitro, *J. Invest. Dermat.*, **4**:325-39, 1941.
 224. PEÑA-CHAVARRIA, A.; GOLDMAN, L.; SAENZ-HERRERA, C.; and CORDERO-CARVAJAL, E. Canities and alopecia in children associated with avitaminosis, *J.A.M.A.*, **132**:570-72, 1946.
 225. PETERS, J. P. Melanuria without melanoma, *Arch. Int. Med.*, **32**:709-17, 1923.
 226. PFEIFFER, C. A.; HOOKER, C. W.; and KIRSCHBAUM, A. Deposition of pigment in the sparrow's bill in response to direct application as a specific and quantitative test for androgen, *Endocrinology*, **34**:389-99, 1944.
 227. PUGH, C. E. M. Tyrosinase from the skin

- of certain black rabbits, *Biochem. J.*, **27**:475-79, 1933.
228. PUGH, C. E. M., and RAPER, H. S. The action of tyrosinase on phenols, *Biochem. J.*, **21**:1370-83, 1927.
 229. RABEN, M. S.; ROSENBERG, I. N.; and ATWOOD, E. B. Fate of other pituitary hormones during purification of corticotropin, *Federation Proc.*, **11**:126, 1952.
 230. RALLI, E. P., and GRAEF, I. Stimulating effect of adrenalectomy on hair growth and melanin deposition in black rats fed diets adequate and deficient in the filtrate factors of vitamin B₁, *Endocrinology*, **37**:252-61, 1945.
 231. RAPER, H. S. The tyrosinase-tyrosine reaction. VI. Production from tyrosinase of 5:6-dihydroxyindole and 5:6-dihydroxyindole-2-carboxylic acid, the precursors of melanin, *Biochem. J.*, **21**:89-96, 1927.
 232. ———. The aerobic oxidases, *Physiol. Rev.*, **8**:245-82, 1928.
 233. RAWLES, M. E. Origin of the mammalian pigment cell and its role in the pigmentation of hair. *In*: GORDON, M. (ed.), *Pigment cell growth*, pp. 1-15. New York: Academic Press, Inc., 1953.
 234. RICHTER, C. P., and CLISBY, K. H. Graying of hair produced by ingestion of phenylthiocarbamide, *Proc. Soc. Exper. Biol. & Med.*, **48**:684-87, 1942.
 235. RILEY, V. T.; HESSELBACH, M. L.; FIOLA, S.; WOODS, M. W.; and BURK, D. Application of chromatography to separation of subcellular enzymatically active granules, *Science*, **109**:361-64, 1949.
 236. RITZ, N. D. Diffuse melanosis, pericardial effusion, and melanuria associated with malignant melanoma: case report with autopsy findings, *Ann. Int. Med.*, **30**:184-95, 1949.
 237. RIVOIRE, R. Le traitement de la maladie d'Addison, *Presse méd.*, **43**:1122-26, 1935.
 238. ROBERT, P. Über die Vitiligo (zugleich ein Beitrag zur Frage der Pigmentbildung), *Dermatologica*, **84**:257-319, 1941.
 239. ROPSHAW, H. S. J. Melanogenesis with special reference to sulfhydryls and protamines, *Am. J. Physiol.*, **103**:535-52, 1933.
 240. ROTH, I., and SUMEGI, I. Pathogenesis of Riehl's melanosis, *Dermatologica*, **100**:163-67, 1950.
 241. ROTHMAN, S. Untersuchungen über die Physiologie der Lichtwirkungen, *Ztschr. f. d. ges. exper. Med.*, **36**:398-446, 1923.
 242. ———. Influence of ascorbic acid on oxidation of tyrosine by ultraviolet light, *Proc. Soc. Exper. Biol. & Med.*, **45**:52-54, 1940.
 243. ———. In vitro studies on pigmentation. I. The oxidation of tyrosine by ultraviolet irradiation, *J. Invest. Dermat.*, **5**:61-67, 1942.
 244. ———. In vitro studies on pigmentation. II. Influence of ascorbic acid on oxidation of tyrosine by ultraviolet irradiation, *ibid.*, pp. 67-74.
 245. ———. Studies on melanuria, *J. Lab. & Clin. Med.*, **27**:687-92, 1942.
 246. ———. Personal communication.
 247. ROTHMAN, S., and FELSHER, Z. The physiology of the skin, *Ann. Rev. Physiol.*, **8**:117-43, 1946.
 248. ROTHMAN, S., and FLESCH, P. Isolation of iron pigment from human red hair, *Proc. Soc. Exper. Biol. & Med.*, **53**:134-35, 1943.
 249. ROTHMAN, S.; KRYSA, H. F.; and SMILJANIC, A. M. Inhibitory action of human epidermis on melanin formation, *Proc. Soc. Exper. Biol. & Med.*, **62**:208-9, 1946.
 250. ROTHMAN, S., and RUBIN, J. Sunburn and para-aminobenzoic acid, *J. Invest. Dermat.*, **5**:445-57, 1942.
 251. ROTHMAN, S.; RUBIN, L.; and HOUSTON, M. Vitiligo, *Arch. Dermat. & Syph.*, **48**:400-410, 1943.
 252. ROWNTREE, L. G., and BROWN, G. B. Tintometer for analysis of color of skin, *Am. J. M. Sc.*, **170**:341-47, 1925.
 253. RUSSEL, W. L. Investigation of the physiological genetics of hair and skin color in the guinea pig by means of the dopa reaction, *Genetics*, **24**:645-67, 1939.
 254. SALLER, K., and MAROSKE, F. Chemische und genetische Untersuchungen an menschlichen Pigmenten, speziell demjenigen des Haars, *Ztschr. f. Konstitutionslehre*, **17**:279-317, 1933.
 255. SCHAAF, F. Manometrische Vergleichsuntersuchungen mit Pressäften aus weisser und pigmentierter Meerschweinchenhaut, *Arch. f. Dermat. u. Syph.*, **176**:646-88, 1938.
 256. SCHAUMANN, K., and DANNEEL, R. Zur Physiologie der Kälteschwärzung beim Russenkaninchen, *Biol. Zentralbl.*, **58**:242-60, 1938.
 257. SCHROEDER, H. Über die Hemmung der Dopareaktion durch das Vitamin C, *Klin. Wchnschr.*, **13**:553-54, 1934.
 258. SCHROEDER, H., and EINHAUSER, M. Über einen Zusammenhang zwischen gestörter

- Vitamin-C-Resorption und pathologischer Pigmentierung bei Gastroenteritis und Achylia gastrica, München. med. Wchnschr., **83**:923-25, 1936.
259. SCHWARTZ, L.; WARREN, L. H.; and OLIVER, E. A. Occupational leukoderma, Weekly Pub. Health Rep., **55**:1111-30, 1940.
 260. SHARLIT, H. Histochemical observations on melanin production in skin, Arch. Dermat. & Syph., **45**:103-11, 1942, and **51**:376-83, 1945.
 261. ———. Cited in discussion of FITZPATRICK (73).
 262. SHEARD, C., and BRUNSTING, L. A. Color of skin as analyzed by spectrophotometric methods. I. Apparatus and procedures, J. Clin. Investigation, **7**:559-74, 1929.
 263. SHEEHAN, H. L. Simmond's disease due to post-partum necrosis of the anterior pituitary, Quart. J. Med., **8**:277-309, 1939.
 264. SINGER, L., and DAVIS, G. K. Pantothenic acid in copper deficiency in rats, Science, **111**:472-73, 1950.
 265. SIZER, I. W. The action of tyrosinase on certain proteins and products of their autolysis, J. Biol. Chem., **169**:303-11, 1947.
 266. ———. The inactivation of invertase by tyrosinase, Science, **108**:335-36, 1948.
 267. SJOLLEMA, B. Kupfermangel als Ursache von Tierkrankheiten, Biochem. Ztschr., **295**:372-76, 1938.
 268. SMITH, S. W., and ELLIS, G. H. Copper deficiency in rabbits; achromotrichia, alopecia and dermatosis, Arch. Biochem., **15**:81-88, 1947.
 269. SODEMAN, W. A. Addison's disease, Am. J. M. Sc., **198**:118-32, 1939.
 270. SPENCER, G. A. Leukoderma produced by anti-oxidants, Arch. Dermat. & Syph., **58**:215-19, 1948.
 271. SPOOR, H. J., and RALLI, E. P. Chemical studies on melanogenesis in normal and adrenalectomized rats, Endocrinology, **35**:325-37, 1944.
 272. STARY, Z. Keratinization of proteins and melanin formation, Bull. faculté méd. (Istanbul), **12**:328-29, 1949.
 273. STEARNER, S. P. Pigmentary studies in the salamander, with especial reference to the changes at metamorphosis, Physiol. Zoöl., **19**:375-404, 1946.
 274. STIRN, F. E.; ELVEHJEM, C. A.; and HART, B. B. The indispensability of zinc in the nutrition of the rat, J. Biol. Chem., **109**:347-59, 1935.
 275. STONE, L. S. Regeneration of neural retina and lens from pigment cells in the eyes of adult salamanders, Zoologica, **35**:23-24, 1950.
 276. SUGIURA, K. The effects of various physical and chemical factors on transplantable mouse melanoma. In: MINER, R. W. (ed.), The biology of melanomas, pp. 369-87. ("Special publications of the New York Academy of Sciences," Vol. 4.) New York: The Academy, 1948.
 277. SULLIVAN, M., and NICHOLLS, J. Nutritional dermatoses in rat; signs and symptoms resulting from diet containing unheated dried egg white as source of protein, Arch. Dermat. & Syph., **45**:295-314, 1942.
 278. SULMAN, F. G. Chromatophorotropic effect of adrenocorticotrophic hormone, Nature, **169**:588-89, 1952.
 279. SUMNER, F. B. Cited by LORUS, J., and MILNE, M. J., How animals change color, Scient. Am., **186**:64-67, 1952.
 280. SZENT-GYÖRGYI, A. Observations on the function of peroxidase systems and the chemistry of the adrenal cortex. Description of a new carbohydrate derivative, Biochem. J., **22**:1387-1409, 1928.
 281. ———. On the mechanism of biological oxidation and the function of the suprarenal gland, Science, **72**:125-26, 1930.
 282. TAUSSIG, J., and WILLIAMS, G. D. Skin color and skin cancer, Arch. Path., **30**:721-30, 1940.
 283. TAYLOR, A. C. Survival of rat skin and changes in hair pigmentation following freezing, J. Exper. Zoöl., **110**:77-112, 1949.
 284. THORN, G. W., *et al.* The clinical usefulness of ACTH and cortisone, New England J. Med., **242**:783-93, 1950.
 285. TODD, T. W.; BLACKWOOD, B.; and BEECHER, H. Skin pigmentation: color top method of recording, Am. J. Phys. Anthropol., **11**:187-204, 1929.
 286. TRINKAUS, J. P. Estrogen, thyroid hormone, and the differentiation of pigment cells in the brown Leghorn. In: GORDON, M. (ed.), Pigment cell growth, pp. 73-92. New York: Academic Press, Inc., 1953.
 287. TROWELL, H. C. Pellagra in African chil-

- dren, *Arch. Dis. Childhood*, **12**:193-212, 1937.
288. TRUEBLOOD, D. V. Malignant melanoma with generalized skin blackening, *North-west Med.*, **46**:199-202, 1947.
289. UNNA, K., and SAMPSON, W. L. Effect of pantothenic acid on nutritional achromotrichia, *Proc. Soc. Exper. Biol. & Med.*, **45**:309-11, 1940.
290. VAN ARMAN, C. G., and JONES, K. K. Formation of dopa from tyrosine by coupled oxidation with ascorbic acid, *J. Invest. Dermat.*, **12**:11-17, 1949.
291. VAN SCOTT, E. J.; ROTHMAN, S.; and GREENE, C. R. Studies on the sulfhydryl content of the skin, *J. Invest. Dermat.*, **20**:111-15, 1953.
292. WARING, H., and LANDGREBE, F. W. Hormones of the posterior pituitary. *In*: PINCUS, G., and THIMANN, K. V., *The hormones*, **2**:498-99. New York: Academic Press, Inc., 1950.
293. WAY, S. C., and LIGHT, S. E. Generalized melanosis. Report of a case with necropsy, *J.A.M.A.*, **94**:241-45, 1930.
294. WELLS, L. J. Pigmentation of the scrotum as a sensitive indicator for androgen, *Anat. Rec.*, **91**:305, 1945.
295. WESTERFELD, W. W. The inactivation of oestrone, *Biochem. J.*, **34**:51-58, 1940.
296. WHEELER, A. H. Unpublished experiments cited by LERNER, A. B., and FITZPATRICK, T. B., *The control of melanogenesis in human pigment cells*. *In*: GORDON, M. (ed.), *Pigment cell growth*, pp. 319-33. New York: Academic Press, Inc., 1953.
297. WHEELER, C. E.; CAWLEY, E. P.; and CURTIS, A. C. The effects of topically applied hormones on growth, pigmentation and keratinization of the nipple and areola, *J. Invest. Dermat.*, **20**:385-99, 1953.
298. WHITAKER, W. L., and BAKER, B. L. Inhibition of hair growth by the percutaneous application of certain adrenal cortical preparations, *Science*, **108**:207-8, 1948.
299. WHITE, A. G. Effect of tyrosine, tryptophane and thiouracil on melanuria, *J. Lab. & Clin. Med.*, **32**:1254-57, 1947.
300. WHITLOCK, F. A. The mechanism of melanin pigment formation in the skin and its relationship to disease. Thesis for the degree of M.D., Cambridge University, April, 1948.
301. WILCOX, J. C. Melanomatosis of the skin and central nervous system in infants. A congenital neuro-cutaneous syndrome, *Am. J. Dis. Child.*, **57**:391-400, 1939.
302. WILKINSON, J. F., and ASHFORD, C. A. Vitamin C deficiency in Addison's disease, *Lancet*, **2**:967-70, 1936.
303. WILLIAMS, C. D. A nutritional disease of childhood associated with a maize diet, *Arch. Dis. Childhood*, **8**:423-33, 1933.
304. WILLIAMS, R. R. Inefficacy of pantothenic acid against the graying of fur, *Science*, **92**:561-62, 1940.
305. WINTERNITZ, R. Einige Versuche und Bemerkungen zur Lehre vom schwarzen Hautfarbstoff, *Arch. f. Dermat. u. Syph.*, **126**:252-66, 1919.
306. WOHLGEMUTH, J. Zur Frage von der Herkunft und der Bildungsstätte des Hautmelanogens, *Schweiz. med. Wchnschr.*, **77**:285-91, 1947.
307. WOODS, M.; DU BUY, H.; and BURK, D. Evidence for the mitochondrial nature and function of melanin granules, *Zoologica*, **35**:30-31, 1950.
308. WRIGHT, L. D., and WELCH, A. D. Folic acid, biotin and pantothenic acid deficiency and the liver storage of various vitamins in rats fed succinylsulfathiazole in highly purified diets, *J. Nutrition*, **27**:55-66, 1944.
309. WRIGHT, P. A. Response of the melanophores of frog skin to pituitary extracts in vitro, *Anat. Rec.*, **96**:540-41, 1946.
310. YAMASAKI, Y. Über die Fermente der Haut, *Biochem. Ztschr.*, **147**:203-15, 1924.
311. ZARAFONETIS, C. J. D. Darkening of gray hair during para-aminobenzoic acid therapy, *J. Invest. Dermat.*, **15**:399-401, 1950.

CHAPTER 23

Enzymes

By AARON BUNSEN LERNER

I. INTRODUCTION	564
II. FACTORS CONTROLLING ENZYME ACTIVITY	565
III. ENERGY-PRODUCING METABOLIC ACTIVITIES	565
A. Glycolysis	566
B. Aerobic Metabolism of Carbohydrate, Fat, and Protein	566
C. Cytochrome Systems	568
D. Oxygen Uptake of Skin	569
E. Enzymes and Vesication	571
F. Conclusions	571
IV. SPECIALIZED METABOLIC ACTIVITIES	572
A. Hyaluronidase	572
B. Proteinases	572
C. Tyrosinase	573
D. Arginase	574
E. Histaminase	574
F. Catalase	575
G. Esterases	575
1. Phosphatase	575
2. Cholinesterase	576
3. Lipase	576
H. Cholesterol-synthesizing Enzymes	577
I. Xanthine Oxidase	577
J. Conclusions	577

I. INTRODUCTION

AN ENZYME may be defined as a protein which acts as a catalyst in the regulation of metabolic processes. Because the rate of metabolic reactions may determine whether a normal or abnormal process occurs, the study of enzymes is an important one.

At the present time it is difficult to correlate the clinical findings of disease states with changes in enzymic activities. For example, in cirrhosis of the liver it is not yet possible to describe the enzyme systems affected or explain how their alteration pro-

duces the various signs and symptoms found in that disease. However, in a few instances, it is possible to explain the mechanism of altered function on the basis of enzyme inhibition. For example, symptoms of parasympathetic stimulation resulting from the administration of drugs, such as diisopropyl fluorophosphate, can be correlated with the inhibition of cholinesterase by the drug, leading to acetylcholine accumulation.

A full understanding of an enzymic process often is not achieved until experimentation has been carried out both in vitro and

in vivo. Frequently the experiment in the test tube does not simulate the physiologic state. In contrast, experiments with the intact animal do not provide sufficient data to reveal the biochemical mechanism of a reaction. Hence several studies must be carried out in order to understand the normal and abnormal physiologic states in man.

In the following discussion the factors which control enzyme activity in general will be presented briefly. Next, enzymes involved in energy-producing reactions within

the cell will be considered. These reactions provide the necessary energy for cells to carry out their special metabolic activities. Finally, enzymes concerned with specialized activities will be discussed. Most of the enzymic activities to be considered are located in the cellular cytoplasm. The nucleus contains relatively little of these activities. Whereas the nucleus seems to be concerned primarily with cellular reproduction, it is in the cytoplasm that reactions occur to supply the energy and compounds necessary for nuclear function.

II. FACTORS CONTROLLING ENZYME ACTIVITY

While all enzymes are proteins, they vary greatly in chemical composition. Some enzymes are shown, on dialysis, to be bound to cofactors or coenzymes. These coenzymes may be heavy metals like zinc, iron, copper, or a derivative of a vitamin, such as riboflavin, niacin, thiamine, or pantothenic acid. To act catalytically, the protein must be present as a complex with its particular coenzyme. Thus enzyme activity is often greatly influenced by the presence of trace metals or vitamin derivatives.

Hormones given *in vivo* can influence the rate of certain metabolic reactions. The mechanism of this type of action is unknown. It has been suggested that hormones exert their influence by acting indirectly and not directly upon the enzymes which catalyze the particular reaction in question.

In the presence of an excess of its substrate and within limits, the rate of a reaction is a direct function of the enzyme concentration:

$$\text{Velocity} = k (\text{enzyme}).$$

Other factors which influence enzymic activity *in vitro* and which may be of great importance in regulating the rates of reactions

in the intact animal are temperature, pH, ionic strength, and oxidation-reduction potentials. Usually the higher the temperature, the faster the rate of reaction. However, when temperatures of about 55° C. are reached, heat denaturation of the enzyme occurs, with a resultant slowing in reaction rate. Most enzymic activities are greatly dependent upon pH. In general, the pH range 6.8–7.4 appears to be optimal for activity, although some enzymic processes require either low or high pH values for optimal action. The ionic strength of the media is frequently an important factor. For numerous enzymic processes involving oxidation and reduction, the oxidation-reduction potential of the system determines the feasibility of a reaction.

In addition to the above factors, some unknown factors are present which regulate enzymic activity in tissues. Some enzymes—for example, tyrosinase and hyaluronidase—exist in tissues in an inhibited or bound state. Under the proper stimulus, such as radiant energy for tyrosinase and inflammation for hyaluronidase, the activities may become very great. The mechanism by which the enzymes are kept in the inhibited state in tissues is not clearly understood.

III. ENERGY-PRODUCING METABOLIC ACTIVITIES

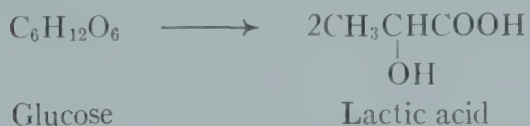
Most of the energy for metabolic function results from the physiologic breakdown of carbohydrate, fat, and protein. Since an

ordinary diet usually contains over 50 per cent carbohydrate, the major portion of the energy requirements of normal tissues, in-

cluding skin, comes from carbohydrate. For this reason carbohydrate metabolism will be considered in greater detail than that of fat and protein.

A. GLYCOLYSIS

By "glycolysis" is meant the physiologic breakdown of carbohydrate to lactic acid. The over-all reaction may be written as follows:



Glycolysis provides energy to cells under anaerobic conditions through the formation of utilizable high-energy phosphate groups. In a large number of metabolic reactions a common reactant, adenosine triphosphate (ATP) or some other phosphate compound, is used. Since much energy is liberated in the hydrolysis of a phosphate group from ATP, the term "high-energy phosphate" is used to designate this group. In the conversion of 1 mole of glucose to lactic acid, there is a net gain of 2 moles of utilizable high-energy phosphate. The energy obtained from these phosphate groups is used for various biologic processes, such as contraction of muscle, activity of sebaceous and sweat glands, and formation of keratin and collagen.

Experimental work done mostly with tissues other than skin—for example, muscle—showed that twelve major chemical reactions occur in the conversion of glycogen via glucose-1-phosphate to pyruvate and then to lactate. Each reaction is reversible, and each is catalyzed by a specific enzyme. Recent evidence points to the occurrence of three of these enzymes in the skin of man and rats, namely, hexokinase, triosephosphate dehydrogenase, and enolase (19, 6). It has been concluded that glycolysis proceeds in skin as it does in other tissues in accordance with the reactions shown in Table 1.

The vitamins riboflavin and nicotinamide serve as cofactors for some of the enzymes having a role in glycolysis.

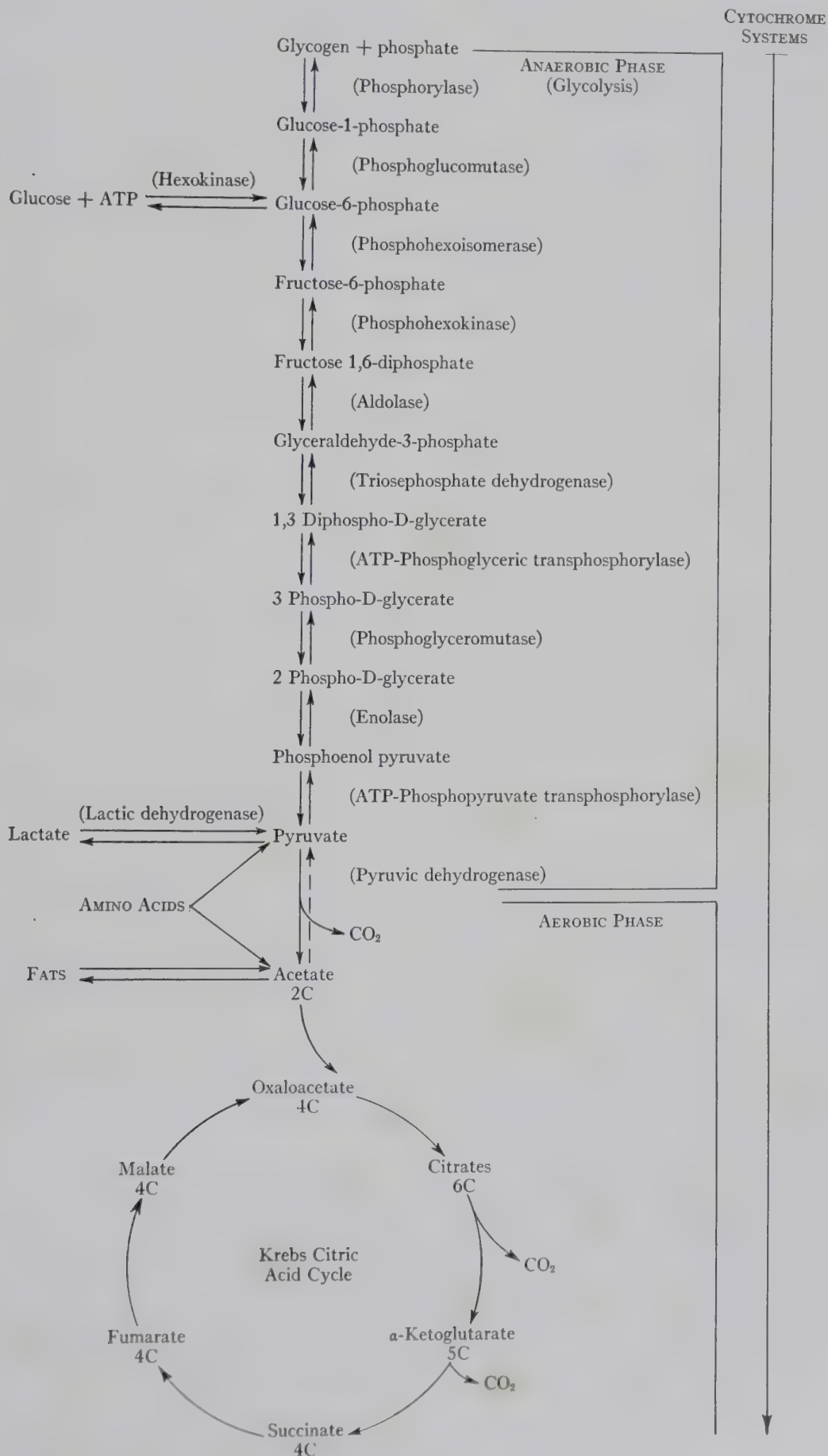
B. AEROBIC METABOLISM OF CARBOHYDRATE, FAT, AND PROTEIN

As seen in Table 1, glycolysis results in the anaerobic conversion of glycogen and glucose to lactate with the production of energy. Next, pyruvate is oxidized by a series of reactions to produce approximately fifteen times more energy than that resulting from glycolysis. At this stage, as well as the following one, in which acetate results from the breakdown of pyruvate, the oxidation of fat and protein follows the same course. That is, fat and protein are partially converted to pyruvate and acetate; and these substances, together with the pyruvate and acetate produced from glycolysis, are oxidized. The sequence of reactions by which pyruvate and acetate are converted to carbon dioxide and water is called the "Krebs citric acid cycle." In this cycle, acetate with its 2 carbon atoms is not oxidized as such but first undergoes condensation with oxaloacetate to form a new compound. The new compound during one complete turn of the cycle is oxidized to 2 molecules of carbon dioxide and water, while oxaloacetate remains as the residue. In other words, the equivalent of 1 molecule of acetate is oxidized while a molecule of oxaloacetate is regenerated at each complete turn of the cycle and is ready to combine with another acetate molecule (see also pp. 473 ff.).

As in the glycolytic reactions, enzymes regulate the rate of each step in the Krebs cycle. Chemical derivatives of the vitamins thiamine, riboflavin, nicotinamide, pyridoxine, and pantothenic acid act as coenzymes in this oxidative phase of metabolism. In some reactions, including those of the glycolytic phase, more than one of these vitamins is involved. This fact may account for the overlapping of clinical signs and symptoms of the avitaminoses.

It would seem that the oxidative metabolism of most animal tissues proceeds via the Krebs citric acid cycle. Because these reactions probably occur in skin, most of the cycle has been outlined in Table 1. Experimental evidence for the occurrence of these reactions in skin, although less extensive

TABLE 1
ENERGY METABOLISM OF CARBOHYDRATES, FATS, AND AMINO ACIDS



than that established for other tissues, consists of the following points:

1. Citric acid, a component of the Krebs cycle, is present in human sweat (33).

2. The addition of pyruvate, succinate, and possibly α -ketoglutarate to rat skin slices increases the oxygen uptake (6). These compounds take part in the cycle.

3. Pyruvic dehydrogenase, a thiamine-containing enzyme required at the beginning of the cycle, is present in animal skin (6, 59, 49). Succinodehydrogenase, a sulfhydryl enzyme required in the cycle, is present in rat skin (6).

4. The vitamins thiamine, riboflavin, nicotinamide, and pantothenic acid, which are coenzymes in the cycle, are present in appreciable amounts in human skin (epidermis and dermis) (34). Their concentration in skin is in the same relative order as in muscle and liver.

On the other hand, Barron *et al.* (6) do not believe that the Krebs citric acid cycle represents the reactions in skin. They suggest, instead, a modified series of reactions linking the oxidation of succinate more directly with that of pyruvate. Their conclusions are based on the following findings:

1. Citrate added to rat skin slices did not increase oxygen uptake, as might be expected if citrate were utilized by skin.

2. Aconitase was detected only in very small amounts with rat skin extracts, and isocitric dehydrogenase was not found at all. These enzymes are required in the cycle (p. 475).

The lack of citrate utilization by skin and the absence of aconitase and isocitric dehydrogenase are negative findings. In the study of intermediary metabolism, definite conclusions cannot be drawn from negative findings because some unknown factor may be present in the tissues which prevents the reaction from occurring. For example, it was once assumed that tyrosinase was not present in human skin because tyrosine did not form melanin in skin slices. Recently it was shown that tyrosinase is present in skin in an inhibited state, and steps must be taken to "activate" tyrosinase so that tyrosine can

be converted to melanin (24). Many similar examples can be found, especially with regard to carbohydrate metabolism. Although Barron may be correct in postulating that the Krebs citric acid cycle does not occur as such in skin, the subject requires further investigation. Until proved otherwise, the most workable hypothesis is that the Krebs cycle represents the main oxidative phase of skin metabolism, as shown in Table 1.¹

C. CYTOCHROME SYSTEMS

All living cells of mammalian origin contain cytochrome systems. The cytochromes function biologically as electron-transporting mechanisms in the oxidative reactions of the Krebs cycle, as well as in many other oxidative reactions, and probably also in phosphate metabolism as related to glycolysis. It is through the cytochrome system that the final step of the reactions takes place. Electrons pass in order through cytochromes b, c, a, and cytochrome oxidase to oxygen. The cytochrome system consists of three distinct parts: (1) the cytochrome pigments, which are heme-protein complexes; (2) the cytochrome reductases, which are enzymes that reduce the cytochrome pigments (some of the cytochrome reductases contain riboflavin compounds); and (3) cytochrome oxidase, an iron- or copper-containing enzyme, or enzymes, which catalyze the oxidation of the reduced cytochrome pigments.

The term "cytochrome reductase" is a general one and refers to anaerobic dehydrogenases of which succinodehydrogenase is an example. The series of reactions shown on the facing page illustrates the mechanism of action of the cytochrome system.

Hydrogen atoms removed from various substrates, such as succinic acid, by specific dehydrogenases are eventually converted to hydrogen ions, through the action of various coenzymes, and to electrons. The electrons are passed through the cytochrome system and eventually operate to convert molecular

1. See a different view represented in chap. 19.

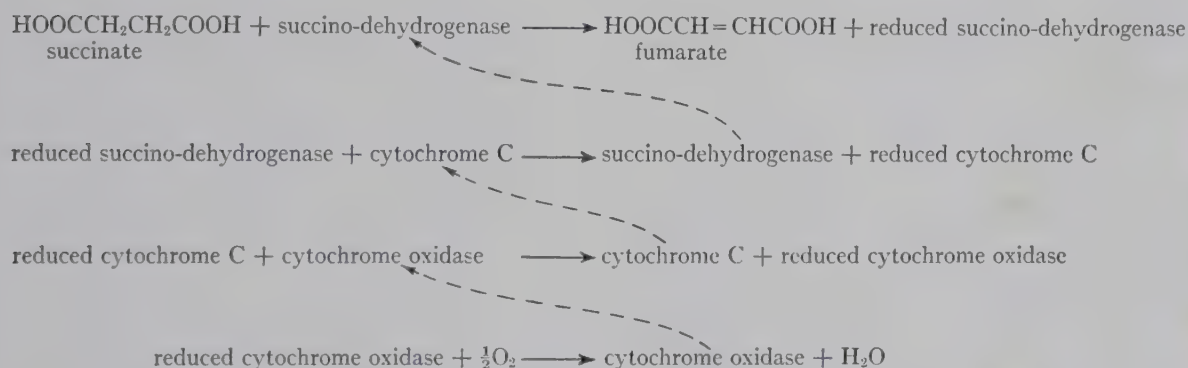
oxygen, in the presence of water, to hydroxyl ions. The oxidations of the Krebs cycle (Table 1) are brought about in this manner.

It has been shown that cytochrome c is decreased about 30 per cent in transplanted squamous-cell carcinoma derived from mouse epidermis by application of methylcholanthrene (15, 16). This is in agreement with the observation that the cytochrome c content of neoplastic tissue is less than that in the tissue of origin (29). However, mouse skin treated with methylcholanthrene showed normal cytochrome c

D. OXYGEN UPTAKE OF SKIN

The determination of oxygen consumption by a given amount of tissue under controlled conditions of temperature, pH, electrolyte concentration, and pressure is a relatively simple measurement which can be carried out on tissue slices or extracts. Several reports have been made concerning the oxygen consumption of human skin slices, as well as those from mice, rats, and rabbits (37, 1, 9, 4, 43). To a large extent the oxygen uptake is a reflection of the Krebs citric acid cycle activity. However, oxygen is utilized in

In the Krebs cycle succinate is converted to fumarate:



Net result: $\text{succinate} + \frac{1}{2}\text{O}_2 \longrightarrow \text{fumarate} + \text{H}_2\text{O}$

values prior to the development of neoplastic tissue.

In late (but not early) hyperplasia produced by methylcholanthrene the cytochrome oxidase content of skin was almost double that of normal and squamous-cell carcinoma. On the other hand, the succinic dehydrogenase content of the squamous-cell carcinoma was twice that of the normal and methylcholanthrene-treated skin.

Cytochrome c from mouse epidermis, unlike that from other tissues, is associated with another substance from which it cannot be separated by adsorption with aluminum oxide (16). It is possible that cytochrome c in human epidermis may also exist in a bound state.

other phases of metabolism, including the oxidation of some amino acids and fats, as well as the completion of glycolysis via the cytochrome system.

The Q_{O_2} (oxygen uptake in cubic millimeters per milligram of dry tissue per hour) for whole rat skin from fetus to 7-day-old animal ranges between 3.6 and 7.3 (6). After 7 days the Q_{O_2} drops abruptly to about 0.8 and remains unchanged thereafter. The Q_{O_2} varies somewhat with the strain of rats as well as with the site from which the skin is obtained. Oxygen is taken up in order of decreasing magnitude from the skin over abdomen, scapula, and sacrum. For fetal human skin the Q_{O_2} was about 1.8 and for adult human skin about 1.6 (6).

TABLE 2

SKIN ENZYMES AND REACTIONS INHIBITED BY VESICATING AGENTS

Vesication Agent	Enzyme and Reaction Inhibited
Nitrogen and sulfur mustards (19, 6, 59, 49)	$\text{C}_6\text{H}_{12}\text{O}_6 + \text{ATP} \xrightleftharpoons{\text{Hexokinase}^*} \text{C}_6\text{H}_{11}\text{O}_5 \cdot \text{PO}_4\text{H}_2 + \text{ADP}$ <p style="text-align: center;">Glucose Glucose-6-phosphate</p>
	$\text{OCHCHOHCH}_2\text{OPO}_3\text{H}_2 \xrightleftharpoons{\text{Triosephosphate dehydrogenase}^*} \text{OCOPO}_3\text{H}_2\text{CHOHCH}_2\text{OPO}_3\text{H}_2$ <p style="text-align: center;">Glyceraldehyde-3-phosphate 1,3 Diphospho-D-glycerate</p>
	Components of Krebs cycle activity as measured by pyruvate and succinate oxidation
Arsenical compounds, e.g., lewisite (19, 6, 59, 49)	$\text{CH}_3\text{COOCH}_2\text{CH}_2\text{N}(\text{CH}_3)_3\text{OH} + \text{H}_2\text{O} \xrightarrow{\text{Cholinesterase}} \text{HOCH}_2\text{CH}_2\text{N}(\text{CH}_3)_3\text{OH} + \text{CH}_3\text{COOH}$ <p style="text-align: center;">Acetylcholine Choline Acetate</p>
	$\text{HOOCCH}_2\text{CH}_2\text{COOH} + \frac{1}{2}\text{O}_2 \xrightarrow{\text{Succinodehydrogenase}^*} \text{HOOCCH=CHCOOH} + \text{H}_2\text{O}$ <p style="text-align: center;">Succinate Fumarate</p>
Iodoacetate (6)	$\text{HOOCCH}_2\text{CH}_2\text{COOH} + \frac{1}{2}\text{O}_2 \xrightarrow{\text{Succinodehydrogenase}^*} \text{HOOCCH=CHCOOH} + \text{H}_2\text{O}$ <p style="text-align: center;">Succinate Fumarate</p>
Methyl bromide (19)	$\text{HOOCCH}_2\text{CH}_2\text{COOH} + \frac{1}{2}\text{O}_2 \xrightarrow{\text{Succinodehydrogenase}^*} \text{HOOCCH=CHCOOH} + \text{H}_2\text{O}$ <p style="text-align: center;">Succinate Fumarate</p>

* Sulfhydryl-containing enzymes. ATP = adenosine triphosphate; ADP = adenosine diphosphate. The terms "pyruvate," "acetate," "succinate," and "fumarate," are used in the reactions, even though the chemical formulae are given for the acids for simplicity. In most cases salts of the acids are present in tissues.

In addition to the decreased oxygen uptake of adult skin as compared with fetal skin, there is a marked decrease in glycolysis—about a fourfold decrease in rat skin and about a two and one-half fold decrease in human skin (6). The decreased oxygen uptake and anaerobic glycolysis of skin from the adult as compared with that of the fetus are possibly due to the presence of relatively inactive connective tissue, collagen, and keratin, which increase with age.

The respiration of whole adult skin is about one-third to one-sixth that of brain, muscle, or liver. However, if allowance is made for the relatively large amount of inactive dermis and stratum corneum, which together make up 80–95 per cent of whole skin, so that only the main cellular portion of the epidermis is considered, skin is approximately two to ten times as active as other tissues. Unfortunately, there are no studies on the oxygen uptake of separated epidermis and dermis. It is likely that the Q_{O_2} of isolated epidermis (which is ordinarily actively proliferating) is significantly higher than the values now given for whole skin.

A correlation of oxygen uptake of skin with physiologic changes has not been advanced except in the study of vesicants. A single report (4) indicates that cosmetic lotions and creams applied to human skin depress the oxygen uptake; but the significance of this finding is not yet known.

E. ENZYMES AND VESICATION²

The relationship of vesicating agents to skin enzymes was studied during World War II. It was thought that a vesicant inhibited some enzyme essential for normal metabolism and that the steps leading to blister formation were initiated by this inhibition. Results found are summarized in Table 2. Most of the toxic vesicants bind sulfhydryl groups. The sulfhydryl-containing enzymes—hexokinase and triosephosphate dehydrogenase of the glycolytic system and succinate dehydrogenase of the Krebs cycle—are inhibited by vesicants (19, 6). In addition, pyruvate oxidation, which probably repre-

sents Krebs cycle activity, is inhibited (59, 6, 49). Hence, it can be seen that vesicating agents block the anaerobic and aerobic phases of carbohydrate metabolism and part of the aerobic phase of fat and protein metabolism. It is hoped that future studies will show how the inhibition of energy metabolism by vesicating agents induces a chain of secondary alterations in metabolism which is associated with separation within the epidermis or with separation of the epidermis from the corium (chap. 28).

F. CONCLUSIONS

There is good evidence that the main steps in energy metabolism in the skin proceed as in other tissues, namely, by glycolysis of carbohydrates; oxidation of the products of glycolysis, fats, and amino acids via the Krebs citric acid cycle; and, finally, a transfer of electrons through the cytochrome system to molecular oxygen. The evidence supporting this conclusion was presented in detail because of the importance of the subject. These energy reactions make possible the specialized metabolic cytoplasmic reactions discussed in the next section, which, in turn, regulate the physiologic processes of skin. Energy obtained from a given compound, such as glucose, is not produced by the direct oxidation of the substance but rather by detailed cyclic processes with numerous checks and balances. In many steps common reactants, such as ATP and cytochrome oxidase, are used in a large number of reactions. The vitamins of the B complex serve as coenzymes for many of the enzymes in these energy reactions. The overlapping of the clinical features of the avitaminoses probably results from the impairment of all reactions past the point where a given vitamin plays its role.

The cellular portion of the skin, exclusive of inert stratum corneum and dermis, is more active in the utilization of oxygen than other tissues. It has not been shown how the activity varies with the different cell types in skin.

Vesicating agents, in general, bind sulfhydryl groups and inactivate enzymes re-

2. See also chap. 28.

quiring such groups for activity. These agents block glycolysis and the Krebs cycle, but the relation of these inhibitions to the

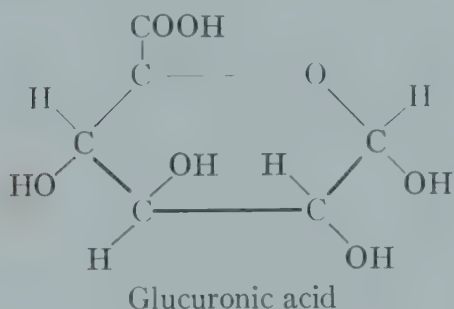
separation of cells or cell layers which leads to the formation of blisters is at present unknown.

IV. SPECIALIZED METABOLIC ACTIVITIES

The normal physiologic state of man depends upon the integration of the specific functions performed by various types of cells. The energy for these metabolic activities is provided by the reactions described earlier. In the following sections certain established enzymic reactions of special interest will be discussed.

A. HYALURONIDASE³

The enzyme hyaluronidase catalyzes the hydrolysis of hyaluronic acid, a viscous mucopolysaccharide with a molecular weight between 200,000 and 500,000, made up of units of glucuronic acid and N-acetyl glucosamine (41).



Antihistaminic agents inhibit hyaluronidase activity and may reduce the dermal response in some inflammatory conditions through their antihyaluronidase effect rather than through their antihistaminic action.

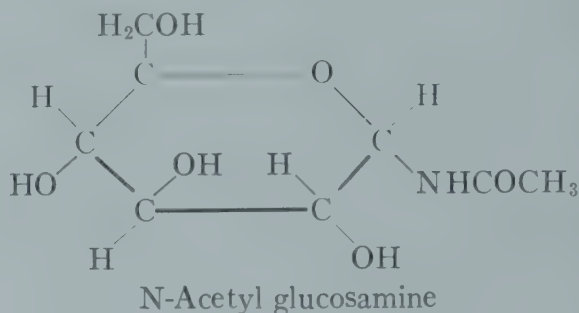
The mucinolytic action of hyaluronidase is markedly increased by nitrogen mustard *in vitro* (17). It has been suggested that mustard may inactivate a natural inhibitor of hyaluronidase.

Because hyaluronidase increases the permeability of skin to many substances, knowledge concerning its relationship to pemphigus has been sought. It was found that intradermal injections of hyaluronidase

in some cases of pemphigus (27) resulted in bulla formation, whereas injection of inactivated hyaluronidase or saline did not. These results were not reproducible consistently. Most of the patients with pemphigus did not show the reaction; and in those who did, not all areas of skin tested gave a positive reaction.

As far as is known, hyaluronidase has no effect on formed collagen. However, recent studies show that this enzyme can bring about the development of abnormally thin fragmented fibers in cultures of chick embryo fibroblasts (38).

The action of hyaluronidase as measured by diffusion of injected India ink is de-



creased in rabbits given one parenteral dose of cortisone (46, 30, 31). Contrariwise, there is an increased diffusion of injected India ink in the skin of rabbits following the local application of cortisone, desoxycorticosterone, or progesterone for 5 months (30, 31).

B. PROTEINASES

It is well known that proteins are formed in the skin. Keratin is constantly being synthesized by epidermal cells. Collagen and elastin are formed, perhaps at a slower rate, by cells in the dermis. Protein catabolism or breakdown parallels the process of synthesis. To carry out these reactions of synthesis and degradation, enzymes must be present in the skin capable of catalyzing the com-

3. This enzyme has been dealt with on pp. 428-33; only a few additional data are listed here.

bination of amino acids and the hydrolysis and degradation of proteins.

A proteolytic enzyme was demonstrated in the skin of man, rabbit, guinea pig, and rat by Beloff, Peters, and Neville-Jones (8, 44). This enzyme is partially extractable from skin and has an optimal activity at pH 7.5. It is different from trypsin and chymotrypsin in specificity. Beloff and Peters (8) and Zamecnik, Stephenson, and Cope (66) showed that a second proteolytic enzyme was present in extracts of rat and dog skin, as well as in blister fluid from human burns. This enzyme is capable of hydrolyzing the synthetic tripeptide, *d,l*-leucyl-glycyl-glycine. It was suggested that this enzyme be called "aminoexopeptidase." Skin proteinase has been shown to liberate Menkin's leucotaxine (p. 100) from proteins (18) (see also p. 704).

Skin proteinase may be associated with vesication. The proteinase, aminoexopeptidase, has been found to show a sharp increase in the lymph drainage from a burned area of a dog's extremity (66). The proteinase content in rat skin decreased after exposure to heat, presumably as a result of the passage of the enzyme into the circulation (8). The proteinase may be released from the skin cells following destruction of the cell membrane so as to initiate the loosening of the epidermis prior to blister formation. This view is consistent with Medawar's findings that digestion of human skin with crude trypsin will result in separation of the epidermis from the dermis (40). The skin proteinase of Beloff and Peters is about 25 per cent inactivated by exposure to 70° C. for 5 minutes—a temperature which usually damages the skin in less than 1 minute. Perhaps heat releases the enzyme in an active form from its normal site. Although these enzymes may have a marked local action in the skin, their release into the general circulation may not be dangerous, because blood contains a specific antiproteinase, which is associated with the most soluble albumin fraction of plasma (7).

It might be well to mention briefly the

collagenases and keratinases, although these enzymes have not been found in human skin. Maschmann⁴ claimed that *Clostridium welchii* elaborates an enzyme which splits collagen. This enzyme, which was named "collagenase," may be associated with the loss of muscular function resulting from collagen destruction in clostridium infections. Collagenase is obtained from culture filtrates of *Cl. welchii*, type A, as well as from other types of clostridia (21, 11, 12). It is said to cause the solution of native collagen, to catalyze the hydrolysis of degraded collagen in hide powder, and to soften muscle. The mere presence of this enzyme in bacteria has been enough to stimulate curiosity concerning its relationship to the dermis in infections and its possible occurrence in normal skin.⁵ Papain is another enzyme which can attack native collagen (14).

Stoughton and Lorincz (58) have demonstrated that normal human serum albumin has an anticollagenase action, whereas the globulin fraction does not. Preliminary studies showed that three patients with lupus erythematosus and one with dermatomyositis had decreased anticollagenase activity in their serum.

Several enzymes obtained from such varied sources as fungi, pineapples, moths, etc., can catalyze the breakdown of keratin from skin, hair, or nails (48, 51) (p. 359).

C. TYROSINASE

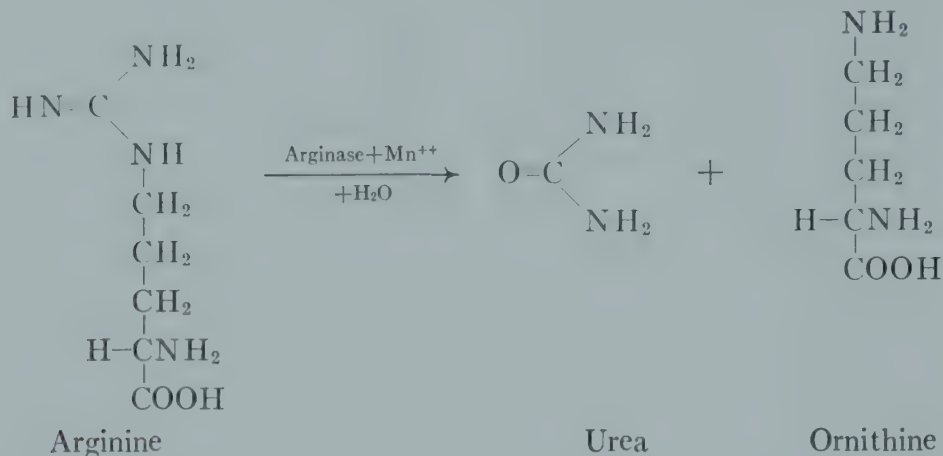
Tyrosinase, which requires copper for action, is present only in the melanocytes of skin, the ciliary body, and some structures of the central nervous system. This enzyme is necessary for normal melanin formation: it catalyzes the oxidation of tyrosine to dopa and then the oxidation of dopa to substances which eventually form melanin (35, 36) (see chap. 22).

4. E. Maschmann, Über Bakterienproteasen. IX. Die Anaerobiase der Gasbranderreger, *Biochem. Ztschr.*, **297**:284–96, 1938.

5. Whether "collagenase" primarily attacks collagen fibrils or ground substance is discussed on pp. 397 and 433.

D. ARGINASE

Arginase, which is found principally in liver, catalyzes the hydrolytic splitting of the amino acid arginine to ornithine and urea. This enzyme requires manganese for activity.



Formerly it was assumed that the urea deposited on the skin in normal and uremic states was a derivative of blood urea. However, when it became known that high concentrations of urea and preformed ammonia were present in human and mouse skin and that human sweat contained urea, arginine, and ornithine, the possibility arose that skin might contain arginase and that urea might be formed directly in skin (54, 56). This possibility became a reality recently, when it was shown that arginase is present in skin, although only in the epidermis, and that the optimum pH for activity was 10—the same as that for liver arginase (47, 39, 52, 55, 62, 63). If human epidermal arginase activity be designated as 100, then that of other animals is as follows: mice 33, rats 7, rabbits 0.7, and turtles 0. Van Scott (62, 63) showed that in human skin nearly all the arginase is present in the prickle- and granular-cell layers, little, if any, activity being found in the basal-cell layer or dermis. Arginase was found in high concentration in sections of skin from warts and in psoriasis lesions, in which conditions the prickle-cell layer is thickened. In seborrheic keratoses and basal-cell carcinomas, in which there is a proliferation of basal cells but not of prickle cells,

arginase was present in low concentration.

Roberts and Frankel (55) compared the arginase content of normal mouse epidermis with mouse squamous-cell carcinoma and with mouse epidermis treated with pure benzene and with benzene containing meth-

ylcholanthrene. Pure benzene produced a slight increase in arginase content. Three paintings with methylcholanthrene in benzene resulted in a marked increase in arginase activity. Subsequent applications of the carcinogen were not so effective, but the arginase content was still greater than normal. Two transplantable squamous-cell carcinomas showed six and eighteen times the arginase content of normal epidermis on a wet-weight basis and eleven and thirty-three times on a total skin nitrogen basis. Arginase was increased also in rat epidermis following the application of the carcinogen dibenzanthracene (39). With low-protein diets, the epidermal arginase activity was increased in both normal and dibenzanthracene-treated rats (39).

E. HISTAMINASE

Little is known concerning histaminase in skin. Skin extracts were said to be able to destroy histamine enzymatically (p. 96). However, in a report by Best and McHenry (10) it was concluded that this action was absent from skin extracts. This problem should be investigated further, especially since it is now possible to prepare better skin extracts than those available earlier.

F. CATALASE

Catalase is an iron-containing enzyme which controls the decomposition of hydrogen peroxide to oxygen and water. It is present in all tissues, including skin, as shown by Wohlgemuth and Szörényi (65).

G. ESTERASES

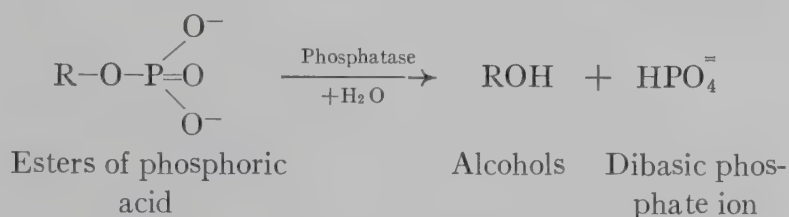
The term "esterase" includes a group of enzymes which catalyze the hydrolysis of esters. Three important esterases have been studied in skin—phosphatase, cholinesterase, and lipase. Each will be considered separately.

1. *Phosphatase*

The phosphatase group pertinent to this discussion includes the enzymes alkaline phosphatase, acid phosphatase, and adenylypyrophosphatase. Because so little work has

tivity was greatest in the papillae. In endothelial cells and in sweat glands the enzyme was concentrated at the cell walls and in the nuclei, with nucleoli showing the greatest activity. The phosphatase activity at the cell walls may indicate that phosphorylating mechanisms play a role in the transport of metabolites in and out of cells.

Fisher and Glick reported that the alkaline phosphatase content of the skin was normal in lupus erythematoses, psoriasis, and papular urticaria (23). Phosphatase activity was increased in proliferating fibroblasts and infiltrates of pyogenic granuloma. The nucleus of the fibroblast gave a more intense reaction than the cytoplasm. In chronic eczema, the cellular perivascular infiltrate of lymphocytes, monocytes, and plasma cells, as well as the fibroblasts, showed increased phosphatase content. The



been done on the last-named enzyme, it will be dispensed with briefly by merely calling attention to the fact that adenylypyrophosphatase activity has been reported in normal and hyperplastic mouse epidermis (53).

Alkaline phosphatase has been studied extensively in the skin by means of the Gomori-Takamatsu histochemical technic. Caution should be exercised in accepting these results, because a recent study (45) showed little alkaline phosphatase in rat liver by direct in vitro assays on isolated nuclei, whereas there was intense staining histochemically. It has been suggested that calcium phosphate formed in the analysis may be adsorbed preferentially by the nuclei during the histochemical procedure. With this point in mind, one questions the studies which demonstrated alkaline phosphatase in the stratum granulosum, capillary endothelial linings, hair follicles, and sweat glands of normal human skin (23, 50). In hair follicles alkaline phosphatase ac-

perifollicular infiltrate of acne vulgaris had increased phosphatase.

Normal mouse skin also contained much alkaline phosphatase activity but no acid phosphatase activity (5). In sebaceous glands of the hamster, acid phosphatase activity was marked, whereas alkaline phosphatase activity was weak (42) (p. 331).

Mouse skin treated with methylcholanthrene showed a marked increase in alkaline phosphatase located in basal cells and connective-tissue cells and was abundant at sites of proliferation (13). Quiescent non-malignant papillomas had little alkaline phosphatase. Whereas acid phosphatase was not present in normal mouse epidermis, it was found to appear and increase with the development of malignancy and was distributed in connective tissue similarly to alkaline phosphatase. Alkaline phosphatase was elevated considerably in healing skin wounds or burns in the rat (22).

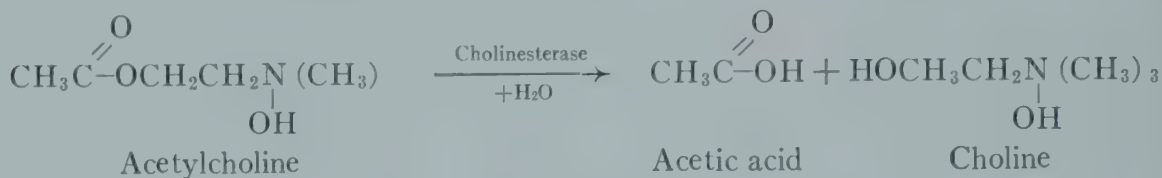
Alkaline phosphatase activity could be

inhibited by very small quantities of beryllium (20, 2). Acid phosphatase required larger quantities for inhibition. Manganese and magnesium could reverse the inhibitory effect. The import of phosphatase inhibition in foreign-body reactions is worthy of further study. The toxic properties of beryllium may result from its competition with manganese and magnesium for enzymes acti-

vated by these metals (20, 2). The phosphatase enzymes may also be related to the deposition of calcium in the skin because there is a reciprocal relationship between the concentration of phosphate and calcium ions in tissues, and phosphatases determine to some extent the concentration of available phosphate ions.

2. Cholinesterase

The enzyme cholinesterase, which is liberated at cholinergic nerve endings, catalyzes the hydrolysis of acetylcholine to acetic acid and choline:



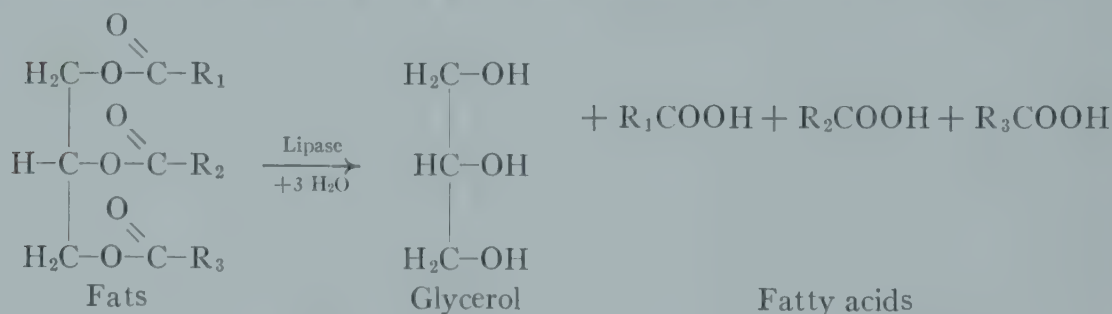
Both rat and human skin contain a specific cholinesterase (61, 60). It is believed that certain disorders of the skin may be associated with abnormal functioning of cholinergic nerves. For example, some cases of urticaria of nervous origin may be brought about by the liberation of acetylcholine in the skin following the stimulation of cholinergic fibers (28). This thesis is supported by the finding that eserine, a powerful in-

hibitor of cholinesterase, acts as an urticariogenic agent in patients with cholinergic urticaria, because eserine causes accumulation of acetylcholine that has been liberated at nerve endings (28) (p. 97).

Thompson (60) found that skin cholinesterase is inhibited in vitro by sodium arsenite and in vivo by certain nonarsenical vesicants of the nitrogen-mustard-gas type.⁶

3. Lipase

The enzymes of the lipase group effect the hydrolysis of esters of fatty acids:



Thompson and Whittaker (61) found that rat and human skin contained lipases capable of hydrolyzing tributyrin and methyl butyrate. Other workers showed that the epidermis of the mouse and the hamster did not contain lipase but that the sebaceous glands and fatty tissue had marked lipase activity (25, 42, 32) (p. 331).

In contrast to the phenomenon that the oxygen uptake of skin decreases with advancing age, lipase activity in rat skin increases with increasing age of the rats. This increase may well be related to changes in sebaceous-gland activity which occur with age.

6. For cholinesterases in and around sweat glands see pp. 163 and 186.

H. CHOLESTEROL-SYNTHESIZING

ENZYMES

It has been known for several years that acetate could be converted to cholesterol by liver extracts. It was found recently that in the rat cholesterol could be formed from acetate in organs other than the liver, but at a slower rate (57). Skin slices were the most active extrahepatic tissue to show this activity (p. 487). Human skin slices also can convert acetate to cholesterol (26).

The formation of cholesterol is a complex process and probably requires the action of several enzymes. It is interesting to speculate on the relation of these systems to the various disorders of fat metabolism in the skin, such as xanthomas found in idiopathic hyperlipemia and hypercholesteremic xanthomatosis.

I. XANTHINE OXIDASE

The conversion of some purine bases to uric acid depends upon the presence of xanthine oxidase. This enzyme requires riboflavin for activity and is found in high concentration in the liver. It is also present in rat skin (64). The activity of xanthine oxidase in human skin may be related to the lesions observed in cases of gout.

J. CONCLUSIONS

The numerous anabolic functions of the skin, such as the formation of keratin, collagen, melanin, sweat, and sebum, as well as the catabolic activities, are controlled by several different types of enzymes. Further knowledge of these enzymic processes will lead to a better understanding of metabolic changes in disease states.

BIBLIOGRAPHY

- ADAMS, P. D. Effect of age on consumption of oxygen and composition of skin of albino rat, *Arch. Dermat. & Syph.*, **36**:606-10, 1937.
- ALDRIDGE, W. N. Beryllium and alkaline phosphatase, *Nature*, **165**:772, 1950.
- ALEXANDER, H. L.; ELLIOT, R.; and KIRCHNER, E. Unresponsiveness of human skin to wheal formation, *J. Invest. Dermat.*, **3**:207-21, 1940.
- AMERSBACH, J. C.; NUTINI, L. G.; and COOK, E. S. Skin respiration as a dermatologic tool, *Arch. Dermat. & Syph.*, **43**:949-55, 1941.
- ANDRES, A. C., and SHERSHUL'SKAYA, L. V. Morphology of phosphorus metabolism in the normal and pathological states. III. Alkaline and acid glycerophosphatases in experimental malignancy of mouse skin, *Arkiv patol.*, **10**:11-23, 1948. Quoted in *Chem. Abstr.*, **43**:5486c, 1949.
- BARRON, E. S. G.; MEYER, J.; and MILLER, L. B. Metabolism of skin; effect of vesicant agents, *J. Invest. Dermat.*, **11**:97-118, 1948.
- BELOFF, A. The skin protease inhibitory factor of plasma, *Biochem. J.*, **40**:108-15, 1946.
- BELOFF, A., and PETERS, R. A. Observations upon thermal burns: the influence of moderate temperature burns upon a proteinase of skin, *J. Physiol.*, **103**:461-76, 1945.
- BERENBLUM, I.; CHAIN, E.; and HEATLEY, N. G. Metabolism of normal and neoplastic skin epithelium; evidence against theory that aerobic glycolysis associated with low R.Q. is feature of disturbed metabolism indicative of tumor growth, *Am. J. Cancer*, **38**:367-71, 1940.
- BEST, C. H., and MCHENRY, E. W. The inactivation of histamine, *J. Physiol.*, **70**:348-72, 1930.
- BIDWELL, E. The K-toxin (collagenase) of *Clostridium welchii*, *Biochem. J.*, **44**:28-32, 1949.
- . Proteolytic enzymes of *Clostridium welchii*; *ibid.*, **46**:589-98, 1950.
- BIESELE, J. J., and BIESELE, M. M. Alkaline phosphatase in mouse skin under methylcholanthrene treatment, *Cancer Research*, **4**:751-55, 1944.
- BRISOU, J., and MILHADE, J. Collagenases, *Bull. Soc. chim. biol.*, **33**:64-68, 1951. Quoted in *Chem. Abstr.*, **45**:8054f, 1951.
- CARRUTHERS, C., and SUNTZEFF, V. Succinic dehydrogenase and cytochrome oxidase in epidermal carcinogenesis induced by methylcholanthrene in mice, *Cancer Research*, **7**:9-14, 1947.
- . Cytochrome c in epidermal carcinogenesis in mice induced by methyl-

- cholanthrene, Arch. Biochem., **17**:261-67, 1948.
17. COSTA, E. Azione di un' azoiprite sulla ialuronidasi, Arch. di sc. biol., **34**:375-87, 1950.
 18. CULLUMBINE, H., and RYDEN, H. N. Study of formation, properties and partial purification of leukotoxine, Brit. J. Exper. Path., **27**:33-46, 1946.
 19. DIXON, M., and NEEDHAM, D. M. Biochemical research on chemical warfare agents, Nature, **158**:432-38, 1946.
 20. DU BOIS, K. P.; COCHRAN, K. W.; and MAZUR, M. Inhibition of phosphatases by beryllium and antagonism of inhibition by manganese, Science, **110**:420-22, 1949.
 21. EVANS, D. G. The production by certain species of *Clostridium* of enzymes disintegrating hide powder, J. Gen. Microbiol., **1**:378-84, 1947.
 22. FELL, H. B., and DANIELLI, J. F. Enzymes of healing wounds; distribution of alkaline phosphomonoesterase in experimental wounds and burns in rat, Brit. J. Exper. Path., **24**:196-203, 1943.
 23. FISHER, I., and GLICK, D. Histochemistry. XIX. Localization of alkaline phosphates in normal and pathological human skin, Proc. Soc. Exper. Biol. & Med., **66**:14-18, 1947.
 24. FITZPATRICK, T. B.; BECKER, S. W.; LERNER, A. B.; and MONTGOMERY, H. Tyrosinase in human skin; demonstration of its presence and of its role in human melanin formation, Science, **112**:223-25, 1950.
 25. GOMORI, G. The microtechnical demonstration of sites of lipase activity, Proc. Soc. Exper. Biol. & Med., **58**:362-64, 1945.
 26. GOULD, R. G. Lipid metabolism and atherosclerosis, Am. J. Med., **11**:209-27, 1951.
 27. GRAIS, M. L. Production of bullae in pemphigus with hyaluronidase, J. Invest. Dermat., **13**:221-22, 1949.
 28. GRANT, R. T.; PEARSON, R. S.; and COMEAU, W. J. Observations on urticaria provoked by emotion, by exercise and by warming the body, Clin. Sc., **2**:253-72, 1936.
 29. GREENSTEIN, J. P.; WERNE, J.; ESCHENBRENNER, A. B.; and LEUTHARDT, F. M. Chemical studies on human cancer. I. Cytochrome oxidase, cytochrome C and copper in normal and neoplastic tissues, J. Nat. Cancer Inst., **5**:55-76, 1944.
 30. HAYES, M. A., and BAKER, B. L. The effect of parenterally administered adrenocortical extract on the intradermal spreading action of hyaluronidase, Endocrinology, **49**:379-83, 1951.
 31. HAYES, M. A., and BRIDGMAN, R. M. Dermal spreading of hyaluronidase as influenced by prolonged local treatment with certain steroid hormones, Proc. Soc. Exper. Biol. & Med., **77**:597-99, 1951.
 32. KUNG, S. K. Lipase activity during experimental epidermal carcinogenesis, J. Nat. Cancer Inst., **9**:435-38, 1949.
 33. LEAKE, C. D. The occurrence of citric acid in sweat, Am. J. Physiol., **63**:540-44, 1923.
 34. LEE, T. H.; LERNER, A. B.; and HALBERG, R. J. Water soluble vitamins in normal human skin, J. Invest. Dermat., **20**:19-26, 1953.
 35. LERNER, A. B., and FITZPATRICK, T. B. Biochemistry of melanin formation, Physiol. Rev., **30**:91-126, 1950.
 36. ———. The control of melanogenesis in human pigment cells. In: GORDON, M. (ed.), Pigment cell growth, pp. 319-33. New York: Academic Press, 1953.
 37. LOEBEL, R. O. Beiträge zur Atmung und Glykolyse tierischer Gewebe, Biochem. Ztschr., **161**:219-39, 1925.
 38. MANCINI, R. E., and SACERDOTE DE LUSTIG, E. Action of hyaluronidase on the mucopolysaccharides of fibroblasts growing "in vitro," Rev. Soc. argent. de biol., **26**:227-33, 1950. Quoted in Chem. Abstr., **45**:8054i, 1951.
 39. MARDASHEV, S. R., and SEMINA, L. A. Arginase in the skin, Biokhimiya, **13**:236-43, 1948. Quoted in Chem. Abstr., **42**:7806, 1948.
 40. MEDAWAR, P. B. Sheets of pure epidermal epithelium from human skin, Nature, **148**:783, 1941.
 41. MEYER, K. The biological significance of hyaluronic acid and hyaluronidase, Physiol. Rev., **27**:335-59, 1947.
 42. MONTAGNA, W., and HAMILTON, J. B. Histochemical analysis of the sebaceous glands of the hamster, Federation Proc., **6**:166-67, 1947.
 43. NEEDHAM, D. M., and DIXON, M. Report to Ministry of Supply, London, 1941. Quoted by PETERS and THOMPSON (49).
 44. NEVILLE-JONES, D., and PETERS, R. A. Further observations on the proteolytic enzymes in rat skin, Biochem. J., **43**:303-8, 1948.

45. NOVIKOFF, A. B. The validity of histochemical phosphatase methods on the intracellular level, *Science*, **113**:320-25, 1951.
46. OPSHAL, J. C. The influence of hormones from the adrenal cortex on the dermal spread of India ink with and without hyaluronidase, *Yale J. Biol. & Med.*, **21**:255-62, 1949.
47. OTTENSTEIN, B. Über Arginase im Hautdialysat und ihre klinische Bedeutung, *Ztschr. f. d. ges. exper. Med.*, **87**:200-207, 1933.
48. PAGE, R. M. Observations on keratin digestion by *Microsporum gypseum*, *Mycologia*, **42**:591-602, 1950.
49. PETERS, R. A., and THOMPSON, R. H. S. The biochemistry of the skin. In: MCKENNA, Modern trends in dermatology, pp. 94-105. New York: Paul B. Hoeber, Inc., 1948.
50. PIRILÄ, V., and ERÄNKÖ, O. Distribution of histochemically demonstrable alkaline phosphatases in normal and pathological human skin, *Acta path. et microbiol. Scandinav.*, **27**:650-61, 1950.
51. POLUNIN, I. An industrial dermatosis due to enzyme action, *Nature*, **167**:442, 1951.
52. ROBERTS, E. Estimation of arginase activity in homogenates, *J. Biol. Chem.*, **176**:213-22, 1949.
53. ROBERTS, E., and CARRUTHERS, C. Adenylpyrophosphatase activity in epidermal carcinogenesis in mice, *Arch. Biochem.*, **16**:239-55, 1948.
54. ROBERTS, E., and FRANKEL, S. Urea and ammonia content of mouse epidermis, *Arch. Biochem.*, **20**:386-93, 1949.
55. ———. Arginase activity and nitrogen content in epidermal carcinogenesis in mice, *Cancer Research*, **9**:231-37, 1949.
56. ROTHMAN, S., and SULLIVAN, M. B. Amino-acids on the normal human skin surface, *J. Invest. Dermat.*, **13**:319-21, 1949.
57. SRERE, P. A.; CHAIKOFF, I. L.; TREITMAN, S. S.; and BURSTEIN, L. S. The extrahepatic synthesis of cholesterol, *J. Biol. Chem.*, **182**:629-34, 1950.
58. STOUGHTON, R. B., and LORINCZ, A. L. Action of collagenase on skin and anti-collagenase factor in human serum, *J. Invest. Dermat.*, **16**:43-52, 1951.
59. THOMPSON, R. H. S. Effect of arsenical vesicants on respiration of the skin, *Biochem. J.*, **40**:525-29, 1946.
60. ———. Action of chemical vesicants on cholinesterase, *J. Physiol.*, **105**:370-81, 1947.
61. THOMPSON, R. H. S., and WHITTAKER, V. P. The esterases of skin, *Biochem. J.*, **38**:295-99, 1944.
62. VAN SCOTT, E. J. Arginase activity in human skin, *Science*, **113**:601-3, 1951.
63. ———. Studies on the arginase activity of the skin, *J. Invest. Dermat.*, **17**:21-26, 1951.
64. WESTERFELD, W. W., and RICHERT, D. A. The xanthine oxidase activity of rat tissues, *Proc. Soc. Exper. Biol. & Med.*, **71**:181-84, 1949.
65. WOHLGEMUTH, J., and SZÖRÉNYI, E. Die Fermente der Haut. X. Über die Katalase der menschlichen Haut. *Biochem. Ztschr.*, **264**:94-103, 1933.
66. ZAMECNIK, P. C.; STEPHENSON, M. L.; and COPE, O. Peptidase activity of lymph and serum after burns, *J. Biol. Chem.*, **158**:135-44, 1945.

CHAPTER 24

Carbon Dioxide Delivery

I. CUTANEOUS RESPIRATION VERSUS GAS EXCHANGE WITH THE ATMOSPHERE	580
II. FACTORS INFLUENCING QUANTITY OF CARBON DIOXIDE DELIVERED TO THE OUTSIDE	581
III. THE PHYSICAL NATURE OF CARBON DIOXIDE DELIVERY	582

I. CUTANEOUS RESPIRATION VERSUS GAS EXCHANGE WITH THE ATMOSPHERE

HUMAN skin takes up oxygen from the atmosphere and gives off carbon dioxide to it. In the early literature this dual process was often referred to as “respiration of the skin.” This expression, of course, is misleading. The actual respiration of the skin is the oxygen uptake and carbon dioxide production of its living cells and is a measure of its vital oxidation processes (10). The gas exchange with the atmosphere is a passive process of diffusion which depends on the difference in gas tension inside the body and the atmosphere, on the permeability of the skin, on its blood flow, and only under special conditions on its own metabolism also (10, 11).

The total oxygen uptake of the skin is much greater than the uptake from the air, and the carbon dioxide production also greatly surpasses the amount given off to the air (10). Thus it is obvious that the bulk of oxygen is supplied to the skin by arterial blood and that most carbon dioxide is given off to the venous circulation (10). However, from the great avidity of the skin in taking up oxygen from the air even at partial oxygen tensions as low as 3–4 mm. Hg (corresponding with 0.5 per cent O₂), it was con-

cluded that the skin utilizes this atmospheric oxygen in addition to that supplied by the blood (13). The “respiratory quotient” of the gas exchange through the skin was found to be from 1.04 to 1.94, with an average value of 1.37 (14). Some features of the uptake of oxygen from the air have been discussed in chapter 3, pages 44–45. The important facts on carbon dioxide delivery are as follows:

If a glass bell, fastened to the skin, is filled with water from above, carbon dioxide will accumulate in the water until its tension equals the carbon dioxide tension in the subdermis (5, 6, 3, 4). To reach an equilibrium takes about 17 hours at room temperatures (6). The final tension of carbon dioxide in the water is about 50 mm. Hg (6). Similarly, carbon dioxide delivery can be demonstrated in the air by surrounding the body or parts of it by a closed chamber and by measuring the increasing carbon dioxide content of the air in the chamber (1, 14).¹

1. With this method Shaw and Messer (13) found the equilibrium established at 70 mm. Hg; Campbell (1) at 40 mm. Hg. The latter value corresponds better with the level of carbon dioxide tension in the tissues (11).

II. FACTORS INFLUENCING QUANTITY OF CARBON DIOXIDE DELIVERED TO THE OUTSIDE

With increasing environmental temperature there is a gradual increase in carbon dioxide delivery to the outside, and this is probably due to gradually increasing cutaneous blood flow. At the critical tempera-

ture gland cells (10, 12, 11). As the activation of eccrine glands is connected with a two to four and a half fold increase of oxygen consumption (9), a similar increase of carbon dioxide production can be expected.

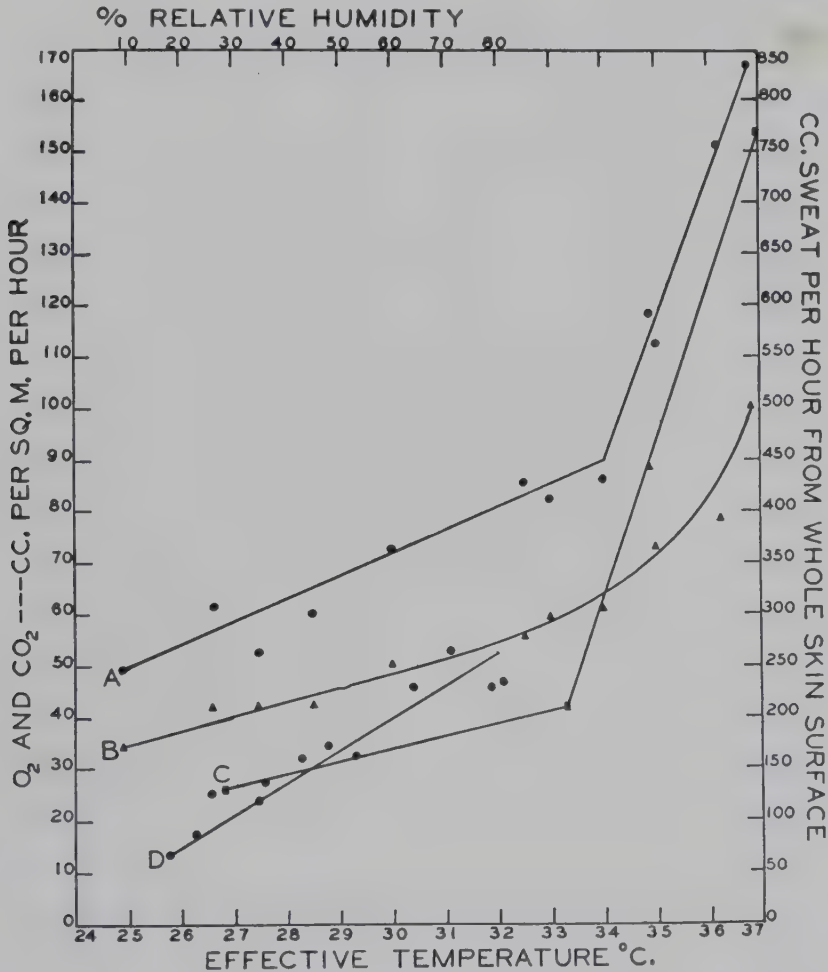


FIG. 1.—Curve A, effect of temperature on rate of carbon dioxide excretion; curve B, effect of temperature on rate of oxygen absorption; curve C, effect of temperature on rate of sweat excretion; curve D, effect of relative humidity on rate of carbon dioxide excretion. From Shaw and Messer (12). (Reproduced by permission of the American Physiological Society.)

ture of sweat outbreak around 34° C. air temperature, there is a sudden dramatic rise (10, 12). Figure 1 shows the coincidence of rise in carbon dioxide delivery (curve A) and outbreak of visible sweating (curve C). Because of the suddenness of the change in carbon dioxide delivery, it is probable that it has something to do with the suddenly increasing metabolism of secretory sweat-

According to older data (10), the total carbon dioxide diffusion through the whole skin surface, exclusive of the head, to the outside in 24 hours is between 7 and 9 gm. carbon dioxide, which is 1.3 per cent of the excretion by the lungs (10). Shaw *et al.* (14) calculated that the "cutaneous respiration" is 1 per cent of the pulmonary respiration.

In the more recent literature the carbon

dioxide diffusion is usually expressed in milliliters per 100 sq. cm. per hour, the volumes being calculated for 0° C. and 760 mm. Hg. Schulze (11) compiled the avail-

TABLE 1

CARBON DIOXIDE DELIVERY THROUGH THE SKIN IN NORMAL INDIVIDUALS (11)

	Room Tempera- ture (° C.)	MICO ₂ / 100 Sq Cm/ Hr
Abernethy (1795).....		1.8
Scharling (1843).....		4.2
Gerlach (1851).....	20	1.1
Reinhard (1869).....	16	0.3
Aubert (1872).....	29.6	0.4
	31.3	0.5
	32	0.6
Röhrig (1872).....	10-11	0.4
	22-28	1.8
Fubini and Ronchi (1878).....	16-20	0.4
	20-24	0.7
	24-30	1.3
Schierbeck (1893).....	29-32	1.1
	34	2.5
Barratt (1897).....	25	0.5
	35	0.9
Von Willebrand (1902).....	20-31.5	0.9
	34	4.2
Campbell (1929).....	35	0.5
Shaw, Messer, and Weiss (1929)	26	0.4
Shaw and Messer (1930).....	25	0.5
	34	0.9
Ernstene and Volk (1932).....	27	1.2
Hediger (1928).....	36*	2.7
Kramer and Sarre (1935).....	34*	0.8
Haussler (1936).....	29*	1.1
Schulze (1943).....	25	0.98

* Temperature of surrounding fluid.

able data. His compilation is reproduced in Table 1.

Arterial hyperemia, produced, for instance, by the electrophoretic introduction

of histamine and acetylcholine (6), increases outward diffusion of carbon dioxide many fold. Also inflammatory conditions, such as ultraviolet erythema (6, 11), psoriasis, and eczematous processes (2), increase carbon dioxide delivery, and it has been assumed that any surplus of carbon dioxide deriving from increased metabolism in inflamed skin is partly discharged to the outside (2). Maximal vasoconstriction produced by electrophoresis of epinephrine reduces the carbon dioxide diffusion to minimum values (6).

Shaw *et al.* (14) and Ernstene and Volk (2) emphasized that there are great individual differences, as has, indeed, been found by most investigators, who explained them by differences in the structure of the skin: carbon dioxide delivery is markedly decreased in persons above forty years of age, and this was thought to be caused by arteriosclerotic and other involutional changes in the skin. It is higher in children than in adults, which might be due to greater thickness of the skin in the adult (11). Remarkably, however, it is very low in the newborn (11). In atrophic-sclerotic processes of the skin it is reduced (8). The diffusion through palms and soles is less than elsewhere; otherwise there are no striking regional differences (11). Pigmented skin seems to be less permeable to carbon dioxide (11), possibly because of densification of cell membranes and other surfaces by ultraviolet light. Carbon dioxide diffusion through the skin is greatly increased in hyperthyroidism, because of increased general metabolism and increased cutaneous blood flow. It is greatly decreased in myxedema (10). Exercise increases carbon dioxide diffusion (10), probably in two ways: by causing sweat secretion and by increasing the carbon dioxide content of the blood.

III. THE PHYSICAL NATURE OF CARBON DIOXIDE DELIVERY

All data confirm the original concept of Krogh (7) that carbon dioxide delivery through the skin surface is a physical diffusion process depending on the difference in

tension between the inside and the atmosphere. Vital processes modify this diffusion to the extent that increased blood circulation promotes the diffusion by delivering

carbon dioxide at a higher speed to the skin, which acts as a diffusion membrane, or to the extent that there are gross structural changes either promoting or hindering diffusion. Only in exceptional cases does the carbon dioxide production of the skin proper contribute to the delivery to the outside; such cases are sudden activation of sweat glands and increased skin metabolism in inflammatory cutaneous processes.

An interesting evidence of the physical nature of oxygen uptake and carbon dioxide delivery has been their reversal under artificially produced experimental conditions. When the carbon dioxide tension of the air is

higher than that of the blood, carbon dioxide is absorbed by the skin; and when the oxygen tension of the atmosphere is lower than that of the blood, oxygen is delivered by the skin to the atmosphere (13). Remarkably enough, venous stasis, with greatly increased carbon dioxide content of the blood, does not increase the diffusion outward (2, 11). This finding has remained unexplained.

Although the skin permits carbon dioxide to pass in an outward direction, it still exerts a considerable barrier effect. If muscle tissue is exposed to air, the rate of carbon dioxide delivery is about fourteen times as rapid as from the skin (14).

BIBLIOGRAPHY

1. CAMPBELL, J. A. Changes in tensions of carbon dioxide and O₂ in gases injected under the skin and into the abdominal cavity, *J. Physiol.*, **59**:1-16, 1924.
2. ERNSTENE, A. C., and VOLK, M. C. Cutaneous respiration in man, *J. Clin. Investigation*, **11**:363-90, 1932.
3. HAEUSSLER, H. Über die Kohlensäurediffusion durch die menschliche Haut, *Arch. f. d. ges. Physiol.*, **237**:448-53, 1936.
4. ———. Über die Kohlensäureaufnahme durch die Haut aus verschiedenen Wässern, *Balneologie*, **3**:217-19, 1936.
5. HEDIGER, S. Experimentelle Untersuchungen über die Resorption der Kohlensäure durch die Haut, *Klin. Wchnschr.*, **7**:1553-57, 1928.
6. KRAMER, K. Untersuchungen über die Kohlensäurediffusion durch die Haut, *Balneologie*, **2**:4-8, 1935.
7. KROGH, A. Some experiments on the cutaneous respiration of vertebrate animals, *Skandinav. Arch. f. Physiol.*, **16**:348, 1904. Quoted by ROTHMAN and SCHAAF (10).
8. MIYAKE, I. Über die Abgabe von Kohlensäure und Wasserdunst durch die Perspiration insensibilis bei einigen Dermatosen, *Jap. J. Dermat. & Urol.*, **24**:7-8, 1924.
9. OHARA, K. Studies on the oxygen consumption of human skin tissues with special reference to that of sweat glands, *Jap. J. Physiol.*, **2**:1-8, 1951.
10. ROTHMAN, S., and SCHAAF, F. *Chemie der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr.*, **1/2**:161-377. Berlin: J. Springer, 1929.
11. SCHULZE, W. Untersuchungen über die Alkaliempfindlichkeit, das Alkalineutralisationsvermögen und die Kohlensäureabgabe der Haut, *Arch. f. Dermat. u. Syph.*, **185**:93-161, 1943.
12. SHAW, L. A., and MESSER, A. C. Cutaneous respiration in man. II. The effect of temperature and of relative humidity upon the rate of carbon dioxide elimination and oxygen absorption, *Am. J. Physiol.*, **95**:13-19, 1930.
13. ———. Cutaneous respiration in man. III. The permeability of the skin to carbon dioxide and oxygen as affected by altering their tension in the air surrounding the skin, *ibid.*, **98**:93-101, 1931.
14. SHAW, L. A.; MESSER, A. C.; and WEISS, S. Cutaneous respiration in man. I. Factors affecting the rate of carbon dioxide elimination and oxygen absorption, *Am. J. Physiol.*, **90**:107-18, 1929.

CHAPTER 25

Biology of Epidermal Cells

By HERMANN PINKUS

I. GENERAL	584
II. REPRODUCTION BY MITOTIC DIVISION	586
III. HYPOTHESES OF CELL CONVERSION	590
IV. BIOLOGICAL EQUIVALENCE OF BASAL CELLS AND PRICKLE CELLS	591
V. RENEWAL TIME	594
VI. OTHER FACTORS AFFECTING MITOTIC ACTIVITY	597
VII. ELIMINATION OF PARTICULATE MATTER	597

I. GENERAL

THE biology of the epidermal cells is determined by their unique position on the exposed surface of the body and by their specific function of partly hindering and partly mediating exchanges between the body and the exterior world. In mammals the epidermis is not protected by an armor of secreted cuticles, as in other phyla, or by massive scales or by mucus, as in lower orders of vertebrates (4); instead, the bodies of the epidermal cells themselves form a protective coat which is constantly shed and renewed. Conditions are further modified in man by the absence of even the relative protection afforded other species by a dense layer of hair. The normal structure and physiologic state of the human epidermis (33, 35, 26, 15, 23) is approximated in fur-bearing animals only in specialized regions, such as the foot pads, or in transient states, such as the first days of life (Fig. 1) of those species which bear naked young (24), or under experimental conditions, when abnormal epidermal hypertrophy is temporarily produced by depilating measures (18).

There are relatively few data available concerning the biology of human epidermis, and data gathered in animal experiments must be treated with a certain amount of reserve because of the different physiologic function of human and animal epidermis.

As compared with small laboratory animals (Fig. 2), the human epidermis (Fig. 3) is much thicker and lacks the support of the many hair follicles which contribute to epidermal regeneration in other species. The human epidermis is a multilayered coat in which there is a constant movement outward from the inside. Undifferentiated germinal cells are transformed into highly specialized, but devitalized, horny flakes by a series of steps involving complicated structural and chemical alterations. All except the basal cells are removed from direct contact with the vascular supply. Exchange of metabolites is maintained by a system of intercellular spaces filled with tissue fluid, which, however, can hardly circulate freely because it is securely sealed off distally. Contact between the cells is maintained by

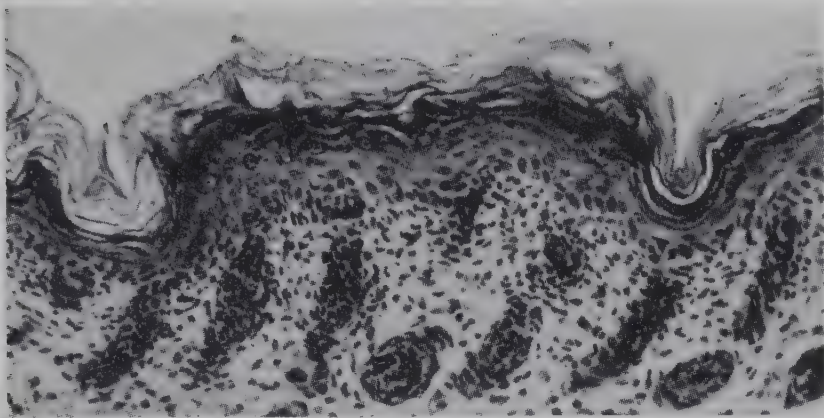


FIG. 1.—Section of skin of a newborn mouse. Thick stratified and heavily keratinized epidermis resembling that of human skin. Incompletely developed hair follicles. $\times 185$.



FIG. 2.—Section of skin of a month-old mouse. Epidermis reduced to one or two layers. Well-developed hairs. $\times 185$.

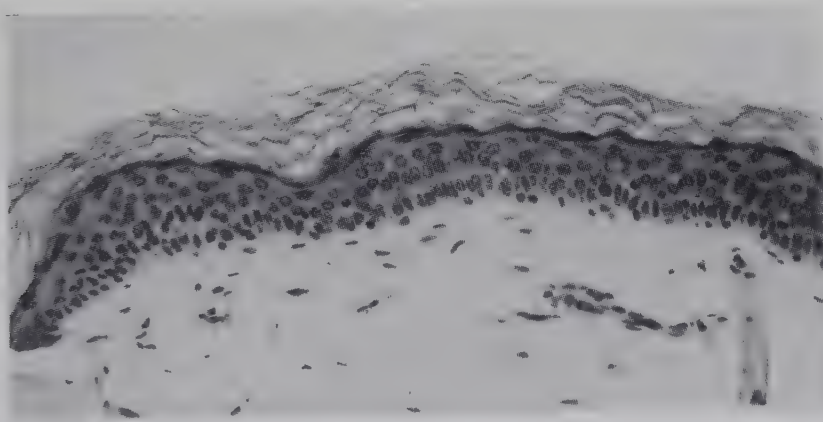


FIG. 3.—Vertical section of normal human skin from the forearm, showing the several strata of the epidermis. $\times 185$.

the system of tonofibrils and intercellular bridges (p. 344), which enables the epidermis to combine a high degree of flexibility with considerable mechanical strength. Little is known concerning the permanence or alterability of these structures, but it seems certain that they limit the movement of individual cells against one another. In addition, the epidermal cells form what may be called a "symbiosis" with extrinsic elements in their midst—such as melanocytes (p. 536), which are derivatives of the neural crest (41); Langerhans cells, which, according to Ferreira-Marques (17), fulfil special

sensory functions; and the lining cells of hair follicles and sweat ducts, which maintain their own integrity and under certain circumstances manifest their biological independence (38). Thus we are dealing with a complicated architectural system in which the very building blocks are in constant flux.

Epidermal metabolism and particularly the process of keratinization are dealt with in other chapters. Here we are concerned with the process of epidermal regeneration and the replacement of the material that is constantly lost from the outer surface.

II. REPRODUCTION BY MITOTIC DIVISION

Tissue cells generally reproduce by mitotic division; and rapidly proliferating epidermis, as in psoriasis and other skin diseases, usually shows numerous mitoses (see Fig. 5). Normal human epidermis, however, contains so few mitoses that time and again other mechanisms have been looked for by investigators who claimed that the small number of mitotic figures was not sufficient to replace the cells lost on the surface.

Mitotic counts in adequately preserved material were first given by Thuringer (45, 46) who reported one mitosis for 2,414 cells on the scalp, for 268,275 cells on the ear, and for 378,325 cells on the leg. These and other authors' figures are given in the form of mitotic indices (mitoses per thousand cells) in Table 1. Most of the indices for adult human epidermis range from 1.6 to 0.1. The extremely low values of Thuringer (46) for leg and ear were obtained on only one specimen in either instance—the leg had been amputated for reasons not stated; the ear came from an executed individual. It appears permissible to disregard these values, because all other observers found mitotic counts of a much higher order of magnitude, similar to those reported by Thuringer for the scalp.

In order to evaluate these figures properly, it would be necessary to know how many cells are lost per unit of time from the normal skin surface. Unfortunately, this is unknown. The only experiment bearing on

this problem is one of Sutton (44), who applied silver nitrate to the skin and found that it required from 7 to 11 days for the stain to wear off. He concluded that this is the time needed for renewal of the stratum corneum, which he estimated to consist of eight layers. Actually, silver nitrate does not penetrate deeply into the horny layer (42). Unpublished observations show that the silver stain can be almost completely removed by stripping away the uppermost four to six layers of an estimated total of fifteen to twenty (39). This variation in rough estimates shows how few quantitative data are known concerning the stratum corneum. It seems safe to assume that, at most, one layer per day is lost under normal circumstances, perhaps only one layer every 2 days, the rate depending on individual and regional differences.

The next point to be considered is the relative area covered by horny cells and basal cells, respectively (Fig. 4). A fully keratinized human epidermal cell is a thin flake with a diameter of from 25 to 30 μ . A basal cell is a cuboidal or columnar body, with a diameter of 5–6 μ . Accordingly, the area of 625–900 μ^2 covered by one horny cell on the surface is occupied by 25 basal cells, each having a basal area of 25–36 μ^2 . This figure does not take into account that the rete ridges and papillae increase the basal area of the epidermis several times over

TABLE 1
MITOTIC INDEX
(Number of Dividing Cells per Thousand Cells)

AUTHOR AND YEAR	MATERIAL	AGE	MITOTIC INDEX
Man, Adult			
Thuringer (1928) (46)	Scalp	Adult	0.41
	Ear	Adult	0.0037
	Leg	Adult	0.0026
Andrew and Andrew (1949) (2) . . .	Antecubital region	Adult	0.13
Katzberg (1952) (28)	Abdominal skin	21-40	Basal 0.48; spinous 0.34
		41-60	Basal 0.73; spinous 0.37
		61-80	Basal 0.76; spinous 0.36
Pinkus (1952) (40)	Forearm, flexor surface	46	1.59*
Man, Growing			
Thuringer (1928) (46)	Prepuce	3 years	0.74
Cooper (1939) (13)	Prepuce	Newborn	1.4-6.8 (av. 3.8)
Broders and Dublin (1939)†	Prepuce	Newborn	7.4
Katzberg (1952) (28)	Abdominal skin	0-20 years	Basal 0.23; spinous 0.26
Mouse			
Carleton (1934)†	Abdominal skin	1-7 days	2-23 (av. 9.8)
Cooper and Franklin (1940) (14) . .	Ear	50 days	0-3.93 (av. 1.45)
Cooper and Reller (1942)†	Ear	2½ months	0.8-1.5
Cowdry and Thompson (1944)† . .	Foot pad	10 days	Basal 25.2; spinous 27.0
Glücksman (1945)†	Interscapular skin	2 months	2.0
Knowlton and Hempelmann (1949)†	Ear	42-56 days	1.69
Knowlton and Widner (1950) (29) .	Ear	42-56 days	0.75
Other Species			
Thuringer (1939)†	Foot pad, cat	Adult	2.37
Henry <i>et al.</i> (1952) (25)	Oral mucosa, rabbit	100 days	5.1
Andrew and Andrew (1949) (2) . .	Back, abdomen, rat	300 days	0.58

* This value was obtained by using certain correction factors not applied by other authors and is considered more reliable by the author. Without correction, the value would be approximately 0.8.

† The references for these are as follows:

- BRODERS, A. C., and DUBLIN, W. B. Rhythmicity of mitosis in the epidermis of human beings, *Proc. Staff Meet. Mayo Clin.*, **14**:423-25, 1939.
- CARLETON, A. A rhythmical periodicity in the mitotic division of animal cells, *J. Anat.*, **68**:251-63, 1934.
- COOPER, Z. K., and RELLER, H. C. Mitotic frequency in methylcholanthrene epidermal carcinogenesis in mice, *J. Nat. Cancer Inst.*, **2**:335-44, 1942.
- COWDRY, E. V., and THOMPSON, H. C., JR. Localization of maximum cell division in epidermis, *Anat. Rec.*, **88**:403-9, 1944.
- GLÜCKSMANN, A. The histogenesis of benzpyrene-induced epidermal tumors in the mouse, *Cancer Research*, **5**:385-400, 1945.
- KNOWLTON, N. P., JR., and HEMPELMANN, L. H. The effect of X-rays on the mitotic activity of the adrenal gland, jejunum, lymph node, and epidermis of the mouse, *J. Cell. & Comp. Physiol.*, **33**:73-92, 1949.
- THURINGER, J. M. The mitotic index of the palmar and plantar epidermis in response to stimulation, *J. Invest. Dermat.*, **2**:313-26, 1939.

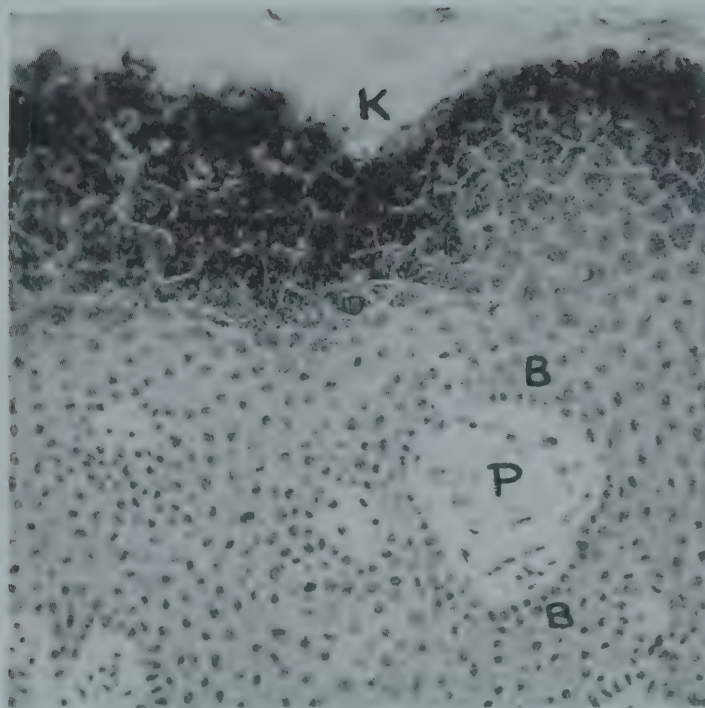


FIG. 4.—Tangential section of normal human epidermis from the ulnar side of the little finger. This section permits direct comparison of the diameters of keratohyalin cells (*K*) and basal cells (*B*), which surround the cross-sectioned papillae (*P*). $\times 185$.

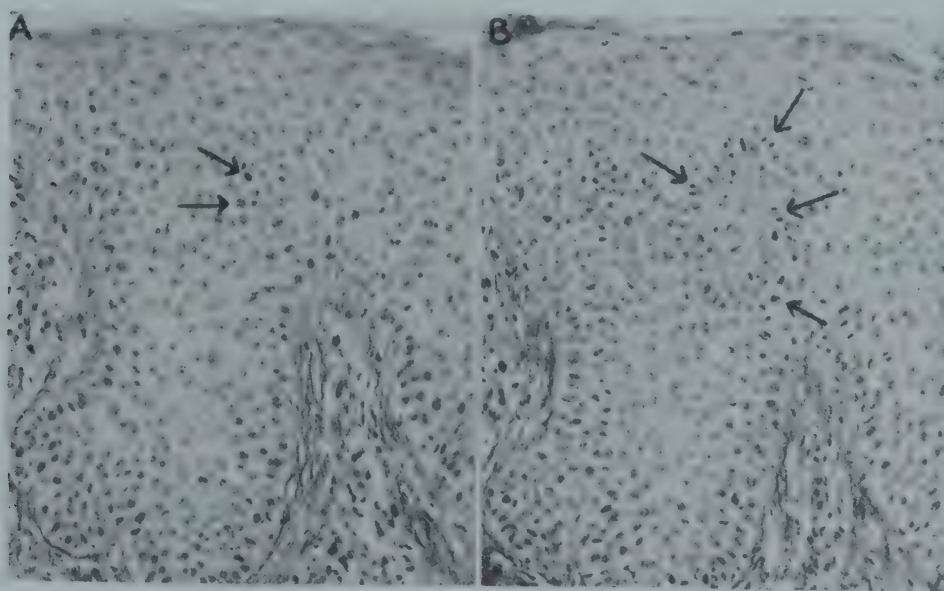


FIG. 5.—Two sections of a series, showing hyperplastic (acanthotic) epidermis in a case of chronic eczematous dermatitis. $\times 140$. *A* shows two mitotic figures (arrows) in the center of a broad rete ridge. Examination of this section alone would suggest that these mitoses are in the upper part of the prickle-cell layer. *B*, a section, $10\ \mu$ distant from *A*, shows the tip of a papilla which occupies the center of the rete ridge. Several mitoses (arrows) are clustered in and near the basal layer, which surrounds the papilla. It is obvious that the mitoses in *A* also are in close proximity to the dermoepidermal junction.

what it would be if the cells were arranged in one plane. As the upper surface of the epidermis is more nearly plane, the ratio of basal cells over keratin cells is increased considerably. If the duration of mitosis is 1 hour (p. 595), then it follows that $1/(25 \times 24)$, or 1 out of every 600 basal cells, has to be found in mitosis at any given time (mitotic index 1.67) in order to replace one horny flake per day under the most unfavorable theoretical circumstances. Actually, a figure of 1:2,000 (mitotic index 0.5)

veals that a mitotic figure that appears to be situated in suprabasal layers in one section actually belongs to the basal layer of a papilla that appears in the following section (Fig. 5). A reinvestigation of this problem appears in order, but it must be admitted that prickle cells are capable of mitotic division. Table 2 lists some values reported in the literature. Amitosis is occasionally suggested in sections of human skin by the presence of deeply indented nuclei. If it does occur, it probably accounts for the presence

TABLE 2
DISTRIBUTION OF MITOSES BETWEEN
BASAL AND SPINOUS LAYERS

AUTHOR AND YEAR	MATERIAL	PER CENT OF MITOSES IN	
		Basal Layer	Spinous Layer
Thuringer (1924) (45).....	Scalp, adult man	12	88
Thuringer (1939)*.....	Foot pad, cat	44.5	55.5
Cowdry and Thompson (1944)†.....	Foot pad, mouse	27.5	72.5
Thuringer and Cooper (1950) (47)...	Abdomen, man	Less than 50	More than 50
	Forearm, man, normal	61	39
	47 hours after complete strip-	56	44
Pinkus (1952)‡ (40).....	72 hours after strip-	44	56
	48 hours after 22 strips	59	41
	32 strips	51	49

* J. M. Thuringer, The mitotic index of the palmar and plantar epidermis in response to stimulation, *J. Invest. Dermat.*, 2:313-26, 1939.
† E. V. Cowdry and H. C. Thompson, Jr., Localization of maximum cell division in epidermis, *Anat. Rec.*, 88:403-9, 1944.
‡ For explanation of these data cf. p. 592.

or even 1:6,000 (mitotic index 0.17) does not seem too low to account for epidermal regeneration by the mechanism of mitotic division. These theoretical values agree well with actual observations as listed in Table 1.
For a long time, it was believed that the basal layer was the sole reproductive layer of the epidermis, the basal cell the only cell capable of mitotic division (35). Thuringer (45) pointed out that mitosis takes place not only in the basal layer but also in prickle cells. He and other authors even reported that three times as many mitoses are found in prickle cells as in basal cells. These statements must be treated with some reserve, as close comparison of serial sections often re-

veals that a mitotic figure that appears to be situated in suprabasal layers in one section actually belongs to the basal layer of a papilla that appears in the following section (Fig. 5). A reinvestigation of this problem appears in order, but it must be admitted that prickle cells are capable of mitotic division. Table 2 lists some values reported in the literature. Amitosis is occasionally suggested in sections of human skin by the presence of deeply indented nuclei. If it does occur, it probably accounts for the presence
of binucleate and multinucleate prickle cells rather than for additional new cells.
Cowdry (15) brought the life-cycle of epidermal cells into analogy with the production of spermatozoa in the testicle, where undifferentiated cells as well as partly differentiated elements undergo division. An even closer analogy is found in the development of anuclear erythrocytes from undifferentiated cells of the bone marrow, as shown in Table 3, which illustrates Cowdry's concept of vegetative intermitotics, differentiating intermitotics, and postmitotics. The comparison of epidermal life-processes with erythropoiesis brings into focus the long-standing argument about whether keratini-

zation is degeneration or differentiation. There is no doubt that the keratin cell is dead, and it has been said that a process ending in cellular death must be called "degeneration." On the other hand, it has been argued that the formation of a highly specialized protein, such as keratin, through a

series of complicated intermediary steps is differentiation. It seems that the two views are compatible, and the example of the anuclear mammalian red cell is another instance of the organism using a devitalized, but highly specialized, end-product for a specific functional purpose.

TABLE 3
COMPARISON OF LIFE-CYCLES OF HUMAN EPIDERMAL
CELLS AND ERYTHROCYTES

Layer		Cell Type	Functional State	Analogon
Stratum germinativum (germinal layer)	Stratum basale (palisade layer, basal layer, germinal layer)	Basal cells, palisade cells	Matrix (vegetative intermitotics)	Stem cell, hemocytoblast
	Stratum spinosum, rete mucosum (prickle-cell layer, Malpighian layer)	Prickle cells, rete cells	Intermediate reproductive layer (differentiating intermitotics)	Normoblast, erythroblast
Stratum granulosum (keratohyalin layer)		Granular cells, keratohyalin cells	Intermediate products of keratinization (postmitotics)	Nucleated red blood cell
Stratum lucidum (eleidin layer)		Eleidin cells		
Stratum corneum (horny layer)		Keratin cells, horn cells	End-product of differentiation	Anuclear red blood cell

III. HYPOTHESES OF CELL CONVERSION

Divergent opinions concerning the biology of the epidermis will be mentioned here mainly for their historical interest. Kromayer (30) maintained for many years and detailed in numerous publications the concept of "desmoplasia," that is, conversion of epithelial cells, particularly in tumors, into connective-tissue cells. Frieboes (20) considered the epidermis as a syncytium without cell boundaries which is permeated by the tonofibrillar system originating in specialized mother-cells of mesodermal character (p. 235). Bostroem (6) contended that the epidermis is continually renewed by conversion of connective-tissue cells into epithelial cells. A somewhat similar view was recently proposed by Levander (31). Andrew and Andrew (2) claimed that lymphocytes which migrate into the epidermis

transform themselves first into clear cells, then into epidermal cells, and that this is the normal mechanism of epidermal regeneration. This hypothesis, which was prompted by the supposedly insufficient rate of epidermal mitosis, appealed to some biologists and immunologists. It seems to be made unnecessary by the calculations shown on the preceding pages. Moreover, the Andrews' (2) conclusions appear not to be sufficiently supported by facts. The translation of the fixed images found in sections of preserved tissues into terms of transformation of one cell type into another is unfortunately wide open to individualistic interpretation. On the basis of material similar to that used by the Andrews (2), it was recently concluded by Andreasen (1) that the fate of lymphocytes in the epidermis is degeneration.

IV. BIOLOGICAL EQUIVALENCE OF BASAL CELLS AND PRICKLE CELLS

One concept which needs more detailed discussion is that of the biological independence of basal cells and prickles cells. First suggested by Thuringer (45), it has recently been elaborated by Eichenlaub and Osbourne (16) and by Thuringer and Cooper (47). According to these authors, the basal layer contains just enough cell divisions to maintain itself, while the supply of cornifying cells is furnished by multiplication of the prickles cells. The significance of this concept is that it explains the fundamental differences in behavior between the relatively be-

the basal and higher layers is best evaluated in the light of evidence furnished by experiments and certain abnormal states. In the epithelization of denuded surfaces, as in healing wounds, the authors agree that it is mainly the lower strata of prickles cells which become mobilized and glide over the denuded surface, while mitotic division takes place some distance from the wound. Usually a thin single layer of flat cells is formed, which have the characteristics neither of basal cells nor of prickles cells. Once the mobilized epidermal cells have covered the



FIG. 6.—Section of embryonic human skin from the side of the foot. A compact layer of cuboidal basal cells is covered by large edematous periderm cells, some of which are exfoliating. $\times 185$.

nign basal-cell cancers and the invasive and metastasizing squamous-cell carcinomas by deriving both from different sources. Thuringer and Cooper (47) based their conclusions on cell counts in human epidermis; Eichenlaub and Osbourne (16) examined embryonic pig skin and found mitoses not only in the inner (basal) layer but also in the outer (periderm) layer of cells. This latter phenomenon was first described in bat embryos by Haeggquist (22), who, however, found that periderm cells are capable of mitosis only in young embryos, while later the stratum basale is responsible for the increasing numbers of prickles cells in the thickening epidermis. The anatomical conditions, as seen in human embryonic skin, are illustrated in Figure 6.

The question of the relationship between

defect, the epidermis again becomes stratified, and the cells which are in contact with the corium assume the character of basal cells (3). On the other hand, if small colonies of epidermal cells are isolated under the conditions of tissue culture (12, 36) (Fig. 7) or of implantation into the subcutaneous tissue of heterologous hosts (48), it is the basal cells which survive and form new layers of prickles cells and keratin. Again, in the epithelization of larger surfaces, such as donor sites of split skin grafts or burned areas, the deep remnants of hair follicles and sweat glands participate actively in the reconstitution of the epidermis. It is thus evident that, at least under temporarily abnormal conditions, all ectodermal derivatives are equivalent in their power of forming keratinizing epidermis.

Even more direct evidence of the interrelationship of basal cells and the higher layers was obtained by removal of part or all of the horny layer of human epidermis by means of stripping with Scotch Tape (40).¹

a burst of mitotic activity in the epidermis (Fig. 8). As high as 4 per cent of all cells capable of mitosis can be found dividing in sections removed 48–72 hours after stripping (Table 4). Under these circumstances ap-

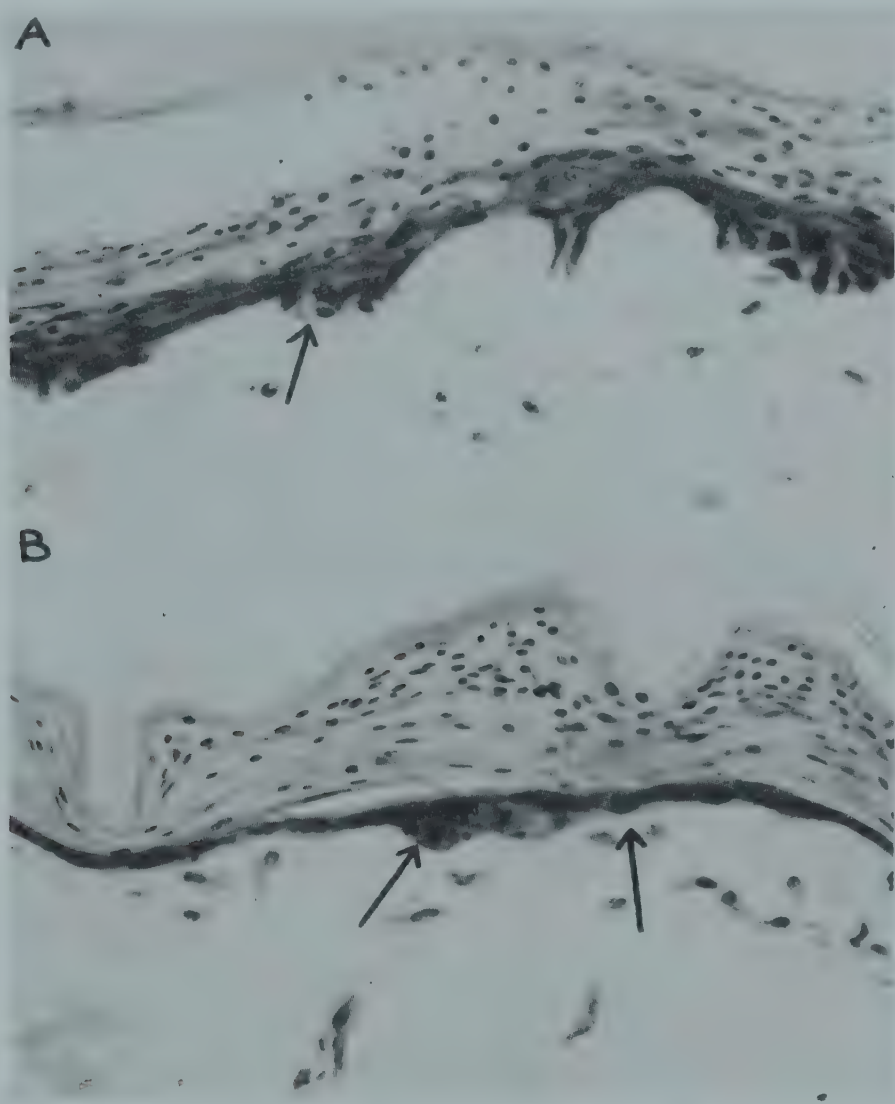


FIG. 7.—Sections through the center of tissue cultures of adult human skin. $\times 280$. *A*, 9-day-old colony shows exfoliation of the upper layers of the epidermis, including keratinized cells and prickles with pyknotic nuclei. The basal layer not only survives but contains numerous mitoses, one of which is shown (arrow), and has begun to form additional layers of cells. *B*, 12-day-old colony shows more complete sloughing of the old cell material, including with the upper layers some pigmented cells, probably basal cells. The corium is covered with several layers of rather flat, "dedifferentiated," actively proliferating cells. Two mitoses are shown. Between these cells and the necrotic old epidermis, there are several layers of parakeratotic flakes. Normal keratinization in human skin is not usually observed under the conditions of tissue culture.

This procedure acts as a powerful stimulus, which, by yet unknown mechanisms, elicits

proximately half the mitotic divisions take place in the basal layer, indicating that this stratum contributes many new prickles cells. Figures are given in Table 2.

1. This method was originally developed by J. Wolf (*Das Oberflächenrelief der menschlichen Haut*, *Ztschr. f. mikr.-anat. Forsch.*, **47**:351–400, 1940).

Katzberg (28) recently found that in the

abdominal skin of young individuals up to the age of twenty years the mitotic index is, on the average, 0.23 in the basal layer and 0.26 in the spinous layer. Both indices rise with advancing age, but the one for the basal layer reaches much higher values. Figures are given in Table 5. Katzberg (28) concluded that the rate of epidermal desquama-

considerable part of the cells in the higher strata. Storey and Leblond (43), studying the influence of temperature on cell division in the foot pads of rats, reported that mitoses occur in the lower third of the stratum germinativum. Figure 9, reproduced from their paper, shows practically all the mitoses in or near the basal layer.

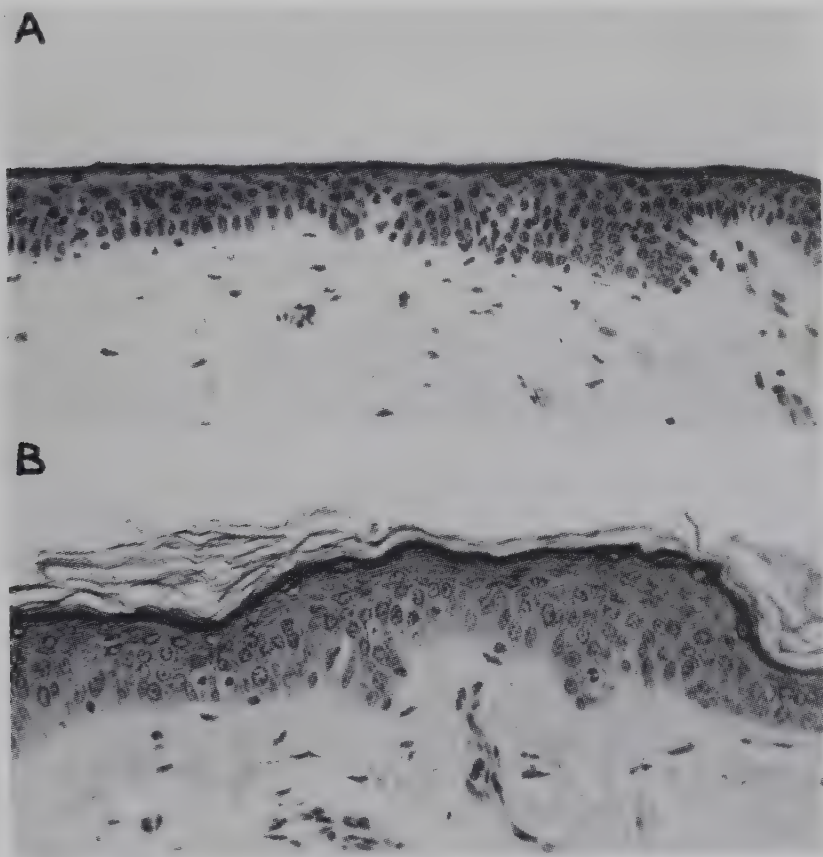


FIG. 8.—Sections illustrating the effect of stripping with Scotch Tape. Skin of the forearm of the same individual whose normal epidermis is shown in Fig. 3. $\times 185$. *A*, $\frac{1}{2}$ hour after complete stripping. Horny layer absent. Early signs of dehydration and pyknosis of numerous nuclei in the prickle-cell layer. *B*, 48 hours after not quite complete stripping. Moderate hypertrophy of the epidermis, with numerous mitoses, mainly in the basal layer. In some places remnants of the old keratin layer are still present. In other places a new keratin layer begins to form above the hypertrophic layer.

tion increases with advancing age and that the basal layer contributes proportionately larger numbers of cells to the higher layers as time goes on. Henry *et al.* (25), working with the oral epithelium of rabbits, found approximately 60 per cent of mitoses in the basal layer and concluded that in this tissue, with its higher rate of desquamation (mitotic index 5.1), the basal layer furnishes a

It would seem obvious from these data that there is no strict separation between basal cells and prickle cells. Even if in quiescent epidermis more cell divisions take place in the higher strata, the various elements are interchangeable in their potentialities, and the general concept of a gradual movement from the basal layer outward appears well established.

V. RENEWAL TIME

It is only in recent years that attempts have been made to estimate the renewal time of the epidermis. Sutton's (44) experiment, using silver nitrate and observing the disappearance of the stain, was mentioned before. Another approach through direct observation was used by von Volkmann (49), who pricked India ink into the epidermis of

tion of mitosis. If the number of cells in a tissue is constant, as one can assume it to be in adult normal skin, the renewal time can be defined as the time needed for the number of cells to double. This can be found by dividing the number of resting cells by the number of dividing cells and multiplying the quotient by the mitotic time.

TABLE 4
NUMBER OF RESTING AND DIVIDING CELLS IN HUMAN EPIDERMIS
STIMULATED BY STRIPPING (40)
EXPERIMENT 1. COMPLETE STRIPPING; TIME VARIABLE

Time (Hours)	Basal- Cell Nuclei	Prickle- Cell Nuclei	All Inter- mitotic Nuclei	Kerato- hyalin Nuclei	Para- keratotic Nuclei	All Post- mitotic Nuclei	All Nuclei	All Mitoses	Per Cent of Inter- mitotics
Normal.	22,400	46,000	68,400	3,300	3,300	71,700	107	0.16
1/2.....	17,500	40,800	58,300	5,400	63,700	193	0.33
12.....	15,900	35,300	51,200	7,300	58,500	107	0.21
24.....	14,200	30,600	44,800	11,200	56,000	44	0.10
36.....	11,900	36,500	48,400	3,100	13,400	16,500	64,900	98	0.20
47.....	10,900	33,400	44,300	9,300	10,900	20,200	64,500	888	2.00
72.....	11,300	37,100	48,400	7,900	20,100	28,000	76,400	1,907	3.94

EXPERIMENT 2. 48 HOURS AFTER VARIABLE NUMBER OF STRIPS

No. of STRIPS	NUMBER OF NUCLEI PER SQUARE MILLIMETER						PER CENT OF INTER- MITOTICS
	Basal Cells	Prickle Cells	All Inter- mitotics	All Post- mitotics	All Nuclei	All Mitoses	
4.....	15,600	31,500	47,100	2,400	49,500	372	0.79
14.....	19,600	28,300	47,900	2,900	50,800	395	0.82
22.....	17,900	30,100	48,000	4,000	52,000	983	1.73
32.....	17,400	28,700	46,100	3,600	49,700	2,156	4.68

the foot pads of guinea pigs and into various regions of human skin. Watching the gradual disappearance of the ink, he found a renewal time of 40-50 days for the thick skin of the guinea pig, 32-36 days for the human hypothenar. A possible objection to these results is the traumatic factor, which may have accelerated the rate of extrusion of the foreign material. Several other authors used indirect methods and calculated renewal time from the mitotic index and the dura-

The latter factor injects an amount of uncertainty into such calculations, because the duration of mitosis has been reported variously as from 15 minutes to 2 hours, the length of time depending somewhat on which stages of the mitotic process were included in the estimate. The matter is further complicated in the epidermis by the two factors that the daughter-cell of a basal cell may divide once more before it reaches the surface and that the number of cells in the

keratin layer is unknown, as nobody has yet devised a method of counting these non-nucleated flakes. Knowlton and Widner (29), who determined mitotic and intermitotic times in mouse tissues by causing mitotic arrest through the use of roentgen rays, found a mitotic time of 30.2 ± 12 minutes for the epidermis of the ear. The intermitotic time was calculated to be 670 ± 300 hours (about 28 ± 12 days). Henry *et al.* (25), using colchicine arrest on the oral epithelium of the rabbit, reported a mitotic time of 64 minutes. While the mitotic time has usually been considered to be a constant, although it may vary from one cell to the next and from one mitotic division to the next following division—at least in

jection of the drug furnishes the actual number of cell divisions which would have taken place during this period; but they did not attempt to include the keratin layer in their calculations.

TABLE 5
LIFE-SPAN OF EPIDERMAL CELLS (28)

AGE (YEARS)	RATIO OF RESTING TO MITOTIC CELLS		LIFE-SPAN IN DAYS = RATIO $\times 0.5 \div 24$	
	Basal	Spinous	Basal	Spinous
0-20. . . .	4,377	3,902	91.2	81.3
21-40. . . .	2,068	2,908	43.1	60.6
41-60. . . .	1,366	2,713	28.5	56.5
61-80. . . .	1,318	2,750	27.5	57.3

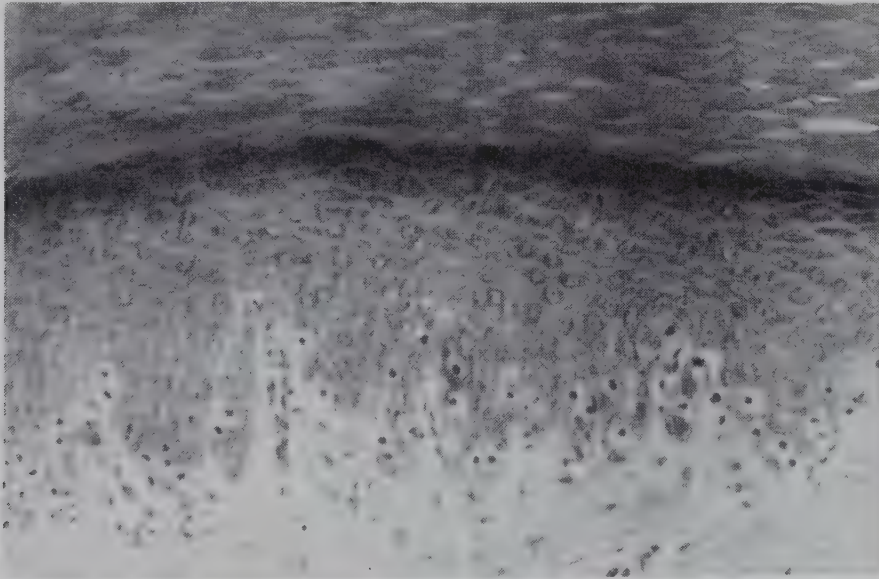


FIG. 9.—Section of plantar skin of a rat, 6 hours after injection of 0.1 mg. of colchicine per 100 gm. of body weight while the animal was kept at 25° – 30° C. A large number of metaphases are visible in the lower region of the stratum germinativum. From Storey and Leblond (43). (Reproduced by permission of Dr. Leblond and the New York Academy of Sciences.)

tissue cultures, which offer the only means of direct observation (32)—it has been reported by Bullough (11) that the mitotic time in female mice varies from 45 minutes to $2\frac{1}{2}$ hours, depending on the stage of the estrus cycle. Storey and Leblond (43) circumvented this difficulty by assuming that colchicine arrests all nuclei entering mitosis during a 6-hour period and that counting the number of metaphases 6 hours after in-

In general, authors were satisfied to estimate the life-span of average cells in the various nucleated layers. Hoffman (27), basing his calculations on Thuringer's figures and the measurement of that author's published photomicrographs, concluded that the average cell remains in the human basal layer for 3,100 hours (129 days), in the spinous layer for 2,900 hours (121 days), and in the keratohyalin layer for 190 hours (7-8

days). These figures appear open to criticism for various reasons, the main one being that Hoffman (27) did not take the third dimension into account when he based his counts and measurements on reproductions of photomicrographs. Katzberg's (28) figures, which were based on actual counts of human

epidermis, are given in Table 5. Both authors arrived at considerably longer renewal times than those observed by von Volkmann (49) on human skin. Similarly, Storey and Leblond's (43) figure of 19.1 days for renewal of rete plus stratum granulosum of the rat's foot pad is much longer than

TABLE 6
SOME FIGURES ON RENEWAL TIME OF EPIDERMAL LAYERS,
LIFE-SPAN OF CELLS, AND INTERMITOTIC TIME
ACCORDING TO VARIOUS AUTHORS

AUTHOR AND YEAR	MATERIAL	BASAL LAYER	SPINOUS LAYER	GRANULAR LAYER	KERATIN LAYER
Man					
Sutton (1938) (44)	Various regions, observed renewal time				7-11 days
Hoffman (1949) (27)	Scalp, renewal time calculated; Thuringer's material	3,100 hours (129 days)	2,900 hours (121 days)	190 hours (7-8 days)	
	Intermitotic time, calculated	3,100 hours	740 hours		
Von Volkmann (1950) (49)	Hypo-thenar } Observed Arm } renewal Forearm } time Thigh } Leg }	Total thickness, 32-36 days			
		Total thickness, 17 days			
		Total thickness, 17 days			
		Total thickness, 30 days			
		Total thickness, 29 days			
Katzberg (1952) (28)	Abdominal skin, calculated life-span				
	0-20 years	91.2 days	81.3 days		
	21-40 years	43.1 days	60.6 days		
	41-60 years	28.5 days	56.5 days		
	61-80 years	27.5 days	57.3 days		
Other Species					
Von Volkmann (1950) (49)	Guinea pig, foot pad, observed renewal time	Combined 1-2 days		5-6 days	Lower two-thirds, 13 days; upper third, 3 weeks
		Total thickness, 6-7 weeks			
Knowlton and Widner (1950) (29)	Mouse, ear, intermitotic time, calculated	670 ± 300 hours (28 ± 12 days)			
Storey and Leblond (1951) (43)	Rat, foot pad, renewal time, calculated	Combined 16.9 days		2.2 days	

von Volkmann's (49) 7 days for the corresponding layers in the guinea pig. It is very likely that the trauma of the latter author's experimental procedure caused accelerated proliferation in the epidermis. It must be realized that "normal" renewal time of the epidermis probably varies within wide limits and is subject to sudden fluctuations caused by minor stimuli (see Table 6). If, as has been shown experimentally, the mere removal of a few superficial keratin layers by the application of four consecutive strips of Scotch Tape can raise the mitotic index from 1.6 to 7.9 (Table 4), then it is

very likely that a good scrubbing with soap and water or increased loss of keratin through friction or other factors may alter the rate of epidermal proliferation considerably.

Depressed mitotic activity in the epidermis may cause a decrease in the rate of cell loss. Recently Bullough and Ebling² found that in adult male mice, maintained on calorically inadequate diets over a 4-week period, epidermal mitotic activity was depressed to 25 per cent of normal without noticeable atrophy resulting. As pointed out by the authors, this must have been due to decreased loss of cells.

VI. OTHER FACTORS AFFECTING MITOTIC ACTIVITY

In addition to minor banal stimulation, variables affecting epidermal mitotic activity are the phenomena of rhythmicity and periodicity. Periodic grouping of mitoses in areas about 100 μ in diameter, as reported by Thuringer (46), was confirmed by other observers. Henry *et al.* (25) showed in the oral epithelium of the rabbit that these areas of high mitotic activity are not fixed but change their places. Cooper (13) described a daily rhythm in the epidermis of human prepuce and of mouse ears. While the peak of mitotic activity in babies was 10:00 P.M., the low between 5:00 and 10:00 A.M., the reverse was true for the mouse (peak at 10:00 A.M., least activity at 10:00 P.M.). Cooper and Franklin (14) suggested

that the difference may be explained by the nocturnal habits of mice. This hypothesis was proved correct by Bullough (7), who could change the peak activity by changing the feeding and resting time of his mice. Other observers confirmed this daily rhythm in man and other species.

Bullough (10) further elucidated the mechanism of epidermal cell activity by showing that increased oxygen tension, increased glucose levels in the blood, and male as well as female hormones favorably influenced the number of epidermal mitoses in mouse skin. The influence of various hormones on epidermis and hair has been the subject of a considerable number of investigations in recent years.

VII. ELIMINATION OF PARTICULATE MATTER

The life-cycle of the epidermal cells, which moves newly formed cells gradually outward, also causes any extraneous matter that enters the epidermis from below to be moved to the surface. To give just one example, it has been shown by Freudenthal (19) that in cutaneous amyloidosis small particles of amyloid often are taken into the epidermis and are then carried to the surface. Erythrocytes have the same fate, while white blood cells, granulocytes as well as lymphocytes, probably add their active motility to this general tendency. Figure 10 shows elastic fibers incorporated in various

layers of the epidermis over an old scar, and it can be assumed that they are being eliminated in this manner. These instances illustrate an unusual mechanism for the excretion of particulate matter onto the body surface, while the loss of fluids is effectively sealed by the obliteration of intercellular spaces in the granular and eleidin layers and by a special barrier action of the lower stratum corneum (5) (p. 32). It may

2. W. S. Bullough and F. J. Ebling, Cell replacement in the epidermis and sebaceous glands of the mouse, *J. Anat.*, **86**:29-34, 1952.

also be mentioned that granular melanin pigment, which is formed by the junctional melanocytes and taken up by the epidermal basal cells, is carried upward and eventually shed with the horny layer. This process is

ment moves from the epidermis downward into the corium and is carried into the regional lymph nodes. Some figures concerning epidermal regeneration are compiled in Table 6.

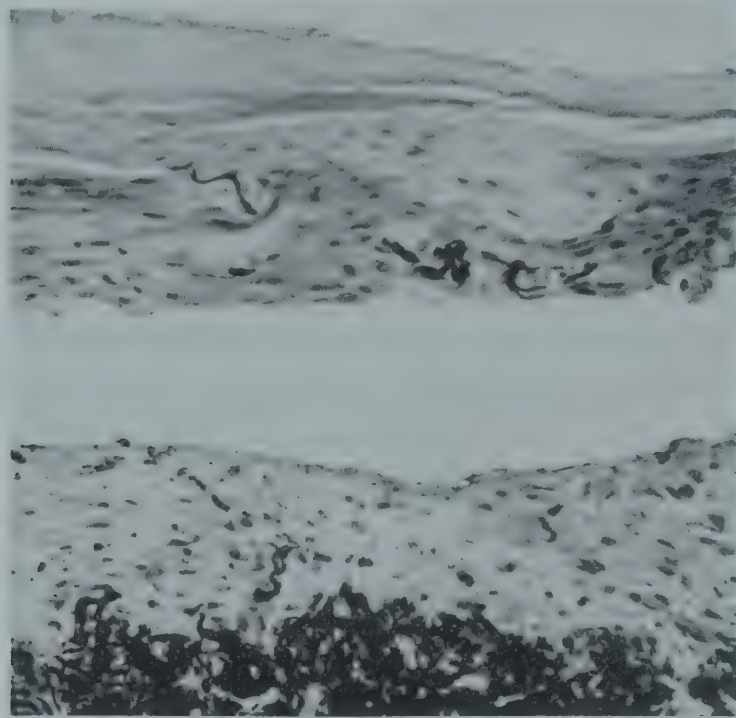


FIG. 10. — Section of human skin from the face, showing an old scar. Elastic fibers are stained black. Fibers are seen in the corium and also incorporated in the epidermis at various levels. The cleft between corium and epidermis is an artifact which occurred during the preparation of the section.

obvious in darkly pigmented skins but can be demonstrated even in light skins by treatment of the horny layer with ferrous salts or silver nitrate (42). On the other hand, and particularly in inflamed skin, pig-

In summarizing this chapter it may be said that recent investigations have added much to our knowledge of epidermal biology, but many facets of this subject remain to be explored.

BIBLIOGRAPHY

1. ANDREASEN, E. On the occurrence of the lymphocytes in the normal epidermis, *Acta dermat.-venereol.* (suppl. 29), **32**:17-21, 1952.
2. ANDREW, W., and ANDREW, N. V. Lymphocytes in the normal epidermis of the rat and of man, *Anat. Rec.*, **104**:217-41, 1949.
3. AREY, L. B. Wound healing, *Physiol. Rev.*, **16**:327-406, 1936.
4. BIEDERMANN, W. Vergleichende Physiologie des Integuments der Wirbeltiere. 1-5, *Ergebn. d. Biol.*, **1**:1-342, 1926; **3**:354-541, 1928; **4**:360-680, 1928; **6**:426-558, 1930.
5. BLANK, I. Factors which influence water content of the stratum corneum, *J. Invest. Dermat.*, **18**:433-40, 1952.
6. BOSTROEM, E. *Der Krebs des Menschen*. Leipzig: Georg Thieme Verlag, 1928.
7. BULLOUGH, W. S. The effects of experimentally induced rest and exercise on the epidermal mitotic activity of the adult male mouse, *Mus musculus* L, *Proc. Roy. Soc. London, s.B.*, **135**:233-42, 1948.
8. ———. The effects of high and low temper-

- ature on the epidermal mitotic activity of the adult mouse, *J. Exper. Biol.*, **26**:76-82, 1949.
9. ———. The relation between epidermal mitotic activity and the blood sugar level of the mouse, *Mus musculus* L, *ibid.*, **26**:83-99, 1949.
 10. ———. Epidermal mitotic activity and oxygen tension, *Nature*, **167**:488, 1951.
 11. BULLOUGH, W. S., and VAN OORDT, G. J. The mitogenic actions of testosterone propionate and of oestrone on the epidermis of the adult male mouse, *Acta endocrinol.*, **4**:291-305, 1950.
 12. CHLOPIN, N. G. Über einige Wachstums- und Differenzierungsercheinungen an der embryonalen menschlichen Epidermis im Explantat, *Arch. f. Entwicklungsmechn. d. Organ.*, **126**:69-89, 1932.
 13. COOPER, Z. K. Mitotic rhythm in human epidermis; introduction and review of literature, *J. Invest. Dermat.*, **2**:289-300, 1939.
 14. COOPER, Z. K., and FRANKLIN, H. C. Mitotic rhythms in the epidermis of the mouse, *Anat. Rec.*, **78**:1-8, 1940.
 15. COWDRY, E. V. A textbook of histology. 4th ed. Philadelphia: Lea & Febiger, 1950.
 16. EICHENLAUB, F. J., and OSBOURNE, R. A. Studies in the histogenesis of the epidermis, *Arch. Dermat. & Syph.*, **64**:700-712, 1951.
 17. FERREIRA-MARQUES, J. Systema sensitivum intraepidermicum. Die Langerhansschen Zellen als Rezeptoren des hellen Schmerzes: Dolorireceptores, *Arch. f. Dermat. u. Syph.*, **193**:191-250, 1951.
 18. FLESCH, P.; KLIGMAN, A. M.; and BALDRIDGE, G. D. An improved method for the separation of the epidermis of laboratory animals, *J. Invest. Dermat.*, **16**:81-84, 1951.
 19. FREUDENTHAL, W. Amyloid in der Haut, *Arch. f. Dermat. u. Syph.*, **162**:40-94, 1930.
 20. FRIEBOES, W. Beiträge zur Anatomie und Biologie der Haut. II and III, *Dermat. Ztschr.*, **31**:57-83 and **32**:1-10, 1920.
 21. GORDON, M. Preface. In: GORDON, M. (ed.), *Pigment cell growth*. New York: Academic Press, Inc., 1953.
 22. HÄGGQUIST, G. Einige Beobachtungen zur Entwicklung der Epidermis, *Arch. f. Dermat. u. Syph.*, **130**:231-40, 1921.
 23. HAM, A. W. *Histology*. Philadelphia: J. B. Lippincott Co., 1950.
 24. HANSON, J. The histogenesis of the epidermis in the rat and mouse, *J. Anat.*, **81**:174-97, 1947.
 25. HENRY, J. L.; MEYER, J.; WEINMANN, J. P.; and SCHOUR, I. Pattern of mitotic activity in oral epithelium of rabbits, *Arch. Path.*, **54**:281-97, 1952.
 26. HOEPKE, H. Die Haut. In: MOELLENDORF, W. VON (ed.), *Handb. d. mikr. Anat. d. Mensch.*, **3**:1:1-116. Berlin: J. Springer, 1927.
 27. HOFFMAN, J. G. Quantitative analysis of the growth of the epidermis, *Arch. Path.*, **47**:37-43, 1949.
 28. KATZBERG, A. The influence of age on the rate of desquamation of the human epidermis, *Anat. Rec.*, **112**:418, 1952.
 29. KNOWLTON, N. P., JR., and WIDNER, W. R. The use of X-rays to determine the mitotic and intermitotic time of various mouse tissues, *Cancer Research*, **10**:58-63, 1950.
 30. KROMAYER, E. Zur Histogenese des Krebsstromas, *Ztschr. f. Krebsforsch.*, **24**:1-9, 1926.
 31. LEVANDER, G. On the epithelium-regeneration in the healing of wounds, *Acta chir. Scandinav.*, **100**:637-49, 1950.
 32. LEWIS, W. H., and LEWIS, M. R. The duration of the various phases of mitosis in the mesenchyme cells of tissue cultures, *Anat. Rec.*, **13**:359-68, 1917.
 33. PATZELT, V. Zum Bau der menschlichen Epidermis, *Ztschr. f. mikr.-anat. Forsch.*, **5**:371-462, 1926.
 34. ———. Histologische und biologische Probleme der menschlichen Haut, *ibid.*, **17**:253-302, 1929.
 35. PINKUS, F. Die normale Anatomie der Haut. In: JADASSOHN, *Handb. d. Haut- u. Geschlechtskr.*, **1**:1:1-378. Berlin: J. Springer, 1927.
 36. PINKUS, H. Über Gewebekulturen menschlicher Epidermis. Ein Beitrag zur Anatomie der Haut, *Arch. f. Dermat. u. Syph.*, **165**:53-85, 1932.
 37. ———. Notes on structure and biological properties of human epidermis and sweat gland cells in tissue culture and in the organism, *Arch. f. exper. Zellforsch.*, **22**:47-52, 1938.
 38. ———. The wall of the intraepidermal part of the sweat duct, *J. Invest. Dermat.*, **2**:175-86, 1939.
 39. ———. Examination of the epidermis by the strip method of removing horny layers.

- I. Observations on thickness of the horny layer and on mitotic activity after stripping, *ibid.*, **16**:383-86, 1951.
40. PINKUS, H. Examination of the epidermis by the strip method. II. Biometric data on regeneration of the human epidermis, *ibid.*, **19**:431-46, 1952.
41. RAWLES, M. E. Origin of melanophores and their role in the development of color patterns in vertebrates, *Physiol. Rev.*, **28**:383-408, 1948.
42. ROTHMAN, S.; RUBIN, L.; and HOUSTON, M. Vitiligo, *Arch. Dermat. & Syph.*, **48**:400-410, 1943.
43. STOREY, W. F., and LEBLOND, C. P. Measurement of the rate of proliferation of epidermis and associated structures, *Ann. New York Acad. Sc.*, **53**:537-45, 1951.
44. SUTTON, R. L., JR. Early epidermal neoplasia; description and interpretation, *Arch. Dermat. & Syph.*, **37**:737-80, 1938.
45. THURINGER, J. M. Regeneration of stratified squamous cell epithelium, *Anat. Rec.*, **28**:31-38, 1924.
46. ———. Studies on cell division in the human epidermis, *ibid.*, **40**:1-13, 1928.
47. THURINGER, J. M., and COOPER, Z. K. The mitotic index of the human epidermis, the site of maximum cell proliferation and the development of the epidermal pattern, *Anat. Rec.*, **106**:255, 1950.
48. TOOLAN, H. W. Proliferation and vascularization of adult human epithelium in subcutaneous tissues of X-irradiated heterologous hosts, *Proc. Soc. Exper. Biol. & Med.*, **78**:540-43, 1951.
49. VOLKMANN, R. VON. Versuche zur Feststellung der Erneuerungsdauer geschichteter Plattenepithelien, *Anat. Nachr.* **1**:86-88, 1950.

CHAPTER 26

Hair Growth

By PETER FLESCH¹

I. FUNCTIONS OF HAIR AND PHYLOGENETIC DECLINE OF HAIR IN MAN	602
A. Mechanical Protection	603
B. Protection against Ultraviolet and Heat Rays	603
C. Thermoregulatory Function	603
D. Disposal of Sweat and Water	603
E. Sexual Function of Hair	603
F. Tactile Function of Hair	603
G. Phylogenetic Decline of Importance in Man	604
II. THE HAIR CYCLE	605
A. Macroscopic Studies of the Hair Cycle	605
B. Microscopic Changes in the Hair Shaft and Hair Follicle during the Hair Cycle	606
C. Artificial Induction of the Hair Cycle	611
D. Changes in the Skin during the Hair Cycle	612
E. The Physiological Controls of the Hair Cycle	614
F. The Human Hair Cycle	615
III. THE RATE OF HAIR GROWTH	616
A. Regional Differences	616
B. Sexual Differences	617
C. Seasonal Variations	617
D. Effect of Age	617
E. Diurnal Variations	617
F. Influence of Thickness of Hair Shaft	618
G. Effect of Cutting and Shaving	618
H. Physical Factors	618
IV. CHANGES IN HAIR WITH AGE	619
V. THE PROBLEM OF NERVOUS FACTORS	621
VI. NUTRITIONAL FACTORS	622
VII. HORMONAL INFLUENCES	624
A. Classification of Hair from the Endocrine Point of View	625
B. Adrenal Cortex	625
C. Pituitary	625
D. Male Sex Hormone	626
E. Ovarian Hormones	626
F. Thyroid	627
G. Pubic Hair	627
H. Axillary Hair	628
I. Beard	629
J. Scalp Hair	630

1. This study was supported by U.S. Public Health Service Grant No. G-3601.

VIII. PATHOPHYSIOLOGY OF ALOPECIAS	631
A. Physiological Alopecias	631
1. Alopecia in the Newborn and Infant	631
2. Hair Loss of Adolescent Boys	631
3. Peroneal Alopecia	632
B. Temporary Alopecia from Chemical Agents	632
1. Thallium	633
2. Unsaturated Lipid-soluble Compounds	633
3. Substituted Amino Acids	634
4. Folic Acid Antagonists and Nitrogen Mustards	634
5. Heparin and Heparinoids	634
C. Alopecia from Ionizing Radiation	634
D. Postinfectious Alopecias	639
E. Possible Hormonal Factors in Alopecia Areata	640
IX. PATHOPHYSIOLOGY OF ESSENTIAL (BENIGN, IDIOPATHIC) HYPERTRICHOSIS IN WOMEN	640
X. EXCRETORY FUNCTION OF HAIR	641
A. Arsenic	641
B. Copper	643
C. Iron	643
D. Zinc	643
E. Lead	644
F. Selenium	644
G. Bromobenzene	644
H. Trace elements	645

I. FUNCTIONS OF HAIR AND PHYLOGENETIC DECLINE OF HAIR IN MAN

HAIR is one of the most characteristic products of mammalian skin. Hairiness has been considered such a distinctive feature that the terms "Trichozoa" or "Pilifera," i.e., animals with hair, have been suggested to denote the mammals. The hairy coat fulfils a number of functions (276).

A. MECHANICAL PROTECTION

The scalp hair forms a protective cushion around the head and minimizes the effects of trauma. This effect is very slight in man; it has been estimated that the hairy covering on 1 sq. cm. of the scalp could support only about 22.5 gm. of weight (26). Hairs in and around openings, like the eyebrows, eyelashes, hairs in the nostrils and external ear canals, protect the structures around which they are located against minor external

harmful influences. Thus the sweat pouring from the forehead is diverted by the eyebrows away from the eyes; vibrissae filter the inhaled air; hairs in the external ear canal are very sensitive to tactile stimuli, such as stray insects or foreign bodies. In the axillae and around the genitalia, the hairs reduce the effect of rubbing by preventing direct contact between two adjacent surfaces. Their function in these areas has been likened to that of roller bearings. However, this protection is of minor importance, because shaving of the axillary vaults does not lead to increased irritation. The low incidence of contact dermatitis on the scalp is probably to a large extent attributable to the protective effect of hair.

In lower animals the protective action of hair and of hairlike epidermal structures is much more pronounced. For example, the

manes of lions offer some protection against claws; the tail hair of cattle acts as a fly swatter; spines and quills provide a formidable armor; etc. (404). The color of the fur and its seasonal changes help in blending animals with their surroundings. In the course of human evolution these defensive devices have completely disappeared, in the same way as pilomotion, a defensive device, has lost its original purpose in man.

B. PROTECTION AGAINST ULTRAVIOLET AND HEAT RAYS

In man, scalp hair provides protection against ultraviolet and heat rays.

C. THERMOREGULATORY FUNCTION

In furred animals the thermoregulatory function is of vital importance. Keratin is a poor heat conductor; this explains the use of woollens and furs for human clothing. Of even greater insulating value are the air layers trapped between the hairs over the body. In the words of McGlone and Bazett, "the hairs allow even exposed areas of the body to have a special environment and almost maintain a 'private climate' similarly to . . . clothed areas" (p. 247). These authors found that the surface of, and the air adjacent to, shaved areas had a lower temperature than hairy areas had (290).

The important role of the hairy coat in conserving heat was strikingly demonstrated by Benedict and Fox (32) when they measured the energy metabolism of normal and hairless mice at various environmental temperatures (Table 1).

It has been stated that the relative importance of piliary insulation in the thermoregulatory system increases with the size of the animal and reaches major proportions in certain species, such as the sheep (210). In man, however, with the disappearance of hair from most portions of the body, hair has lost its protective function against cold, with the exception of scalp hair. Phylogenetically, the loss of a natural fur coat in man had gone hand in hand with the development of eccrine sweat glands over the entire skin surface and with an efficient

nervous and humoral control of the sweat apparatus. Thereby man has acquired protection against severe heat far superior to that of other mammals (381) (p. 180).

D. DISPOSAL OF SWEAT AND WATER

The hairy covering with its large surface promotes the evaporation of sweat and helps in draining water from the skin after bathing or following exposure to rain. In the tree-dwelling anthropoid apes (orang) the hair slopes have a direction favoring the

TABLE 1
ENERGY METABOLISM OF NORMAL AND
HAIRLESS MICE (32)

Zone of Thermal Equilibrium*	Normal Mice (28° C.)	Hairless Mice (34° C.)
Cal/sq meter/24 hr produced at zone of thermal equilibrium	640	750
Heat production at about 16° C.	935	Ca. 2,250

* Zone of thermal equilibrium is the temperature at which the metabolic heat is dissipated into the environment at the rate of its production (chap. 10).

drainage of water, and the fur serves as a raincoat (403). Originally, the human beard may have served the same purpose (404).

E. SEXUAL FUNCTION OF HAIR

The function of hair in sexual life is apparent when we consider that even a hairless mammal, like man, has retained hair in areas of sexual importance. In the axillary and pubic regions, hair promotes the evaporation of apocrine sweat and provides sexual attraction; the pubic hair also receives and transmits tactile stimuli during sexual activity. Magical beliefs in the power and importance of hair, as exemplified by biblical stories and common superstitions, have their origins in this primitive sexual function (23).

F. TACTILE FUNCTION OF HAIR

Hair is a tactile organ. It acts like a lever, magnifying and transmitting touch stimuli

to the rich nervous plexuses around the hair follicles. In man, touching of the eyelashes provokes a lid reflex; tactile stimulation of hairs in the external ear canal of cats provokes a violent scratch reflex (p. 138). Removal of the hairs raises the tactile threshold. Thus on 9 sq. mm. of a shaved skin surface the minimum stimulus required to elicit a tactile sensation was found to be 36 mg.; on the same surface, before it was shaved, 2 mg. was effective (409). The whiskers of animals are important tactile organs. Like the antennae of insects, they supplement vision and transmit tactile information about widths of pathways which the animal can pass with safety.

TABLE 2
DENSITY OF HAIR IN PRIMATES (385)
(Average Density of Hair/Sq Cm)

Species	Scalp	Back	Chest
Platyrrhine monkeys	1,852	1,737	610
Gibbons.....	2,035	1,727	699
Anthropoid apes....	307	276	90
Man.....	312	0	1

From the above enumeration it is obvious that hair is not essential for the well-being of the human species. In sharp contrast, hair is essential for lower animals in their struggle for survival. As a rule, naked mutants of furred animals are biologically inferior and less viable than their hairy counterparts, although this may be due, at least partly, to other concomitant biological defects. In man, in the course of evolution, the hairy coat became reduced to the point of partial disappearance from the major part of the body surface, and human hair has become a rudimentary organ.

G. PHYLOGENETIC DECLINE OF IMPORTANCE IN MAN

The reduction of the number of hairs in the course of evolution has its beginning in

the anthropoid apes. Hair loss in man may be considered to be the most extreme manifestation of an evolutionary trend. The painstaking data of Schultz on the density of hair in primates well illustrate this trend (Table 2) (385). In this case, too, human ontogeny repeats phylogeny. The densities of hair in 6-month-old fetuses of man and anthropoid apes are very similar; at this age, man may be even more hairy than the anthropoid apes. In the newborn human infant the number of hairs is already greatly reduced (295).

It is not known what gave rise to the hairless condition of man. Vivid imaginations have attributed the hair loss to man's superior brain power, which made the discovery of fire possible, enabled him to seek shelter in caves, and thus made hair unnecessary (403). Hairless man may have had an advantage in his fight against vermin. The possibility of temporary hair loss during lactation, as occurs in other species, has also been considered; such loss of hair would have prevented matting of the hair during breast feeding (360). Whatever the cause, it is probably safe to speculate that the original hairless man was a mutant (91). Naked mutants have been described in a large variety of species (436), such as the fowl (100), mouse (91, 102, 82, 104), rat (102, 363), rabbit (102, 69), horse (436), cattle (90), dog (263), cat (264), and swine (103).² Although most of the mutants are biologically inferior, some, like the Mexican hairless swine, are normal and healthy in all respects. In man the hairless condition, together with a more efficient thermoregulatory system, may have made it possible for him to thrive in hot climates and was no obstacle in the way of adapting himself to cold temperatures by clothing.

2. For additional references on hairless species consult Thigpen (436) and Roberts *et al.* (363).

II. THE HAIR CYCLE

With the possible exception of a few species, like the Angora rabbit and sheep (122), in most animals the hair follicles do not produce hair continuously. Periods of activity alternate with periods of rest. During these stages the hair follicles, hair shafts, and, in lower animals, the other appendages and layers of the skin undergo characteristic changes, commonly referred to as the "hair cycle." The hair cycle may be defined as the period which begins with the formation and growth of a new hair, followed by a resting stage, and ending with the growth of another new hair from the same follicle. To understand this process, it is necessary to present a brief outline of the morphologic aspects of the events occurring during the growth and replacement of the hair.

A. MACROSCOPIC STUDIES OF THE HAIR CYCLE

The replacement of hair in animals may be studied in a number of ways.

1. In albino animals the most commonly used method consists of close clipping of the fur, with subsequent observation of the rate and regions of regrowth. Shaving of the skin is less suited for such studies, as it may lead to epidermal damage, with disturbance in the normal sequence of hair cycles (p. 611).

2. Another method of visualizing the replacement of hair is by dyeing the albino fur with a permanent stain, like picric acid (244) or proflavine (122). The newly grown white hair stands out in sharp contrast to the old yellow-colored coat. An ingenious technic of systemic administration of a dye (9-phenyl-5,6-benzoisoalloxazine) has been developed by Haddow *et al.* for the demonstration of the newly grown fur of albino rats. The orange-yellow pigment is taken up by the newly formed hair, presumably because of the increased vascularity in the area of rapid growth (193).

3. The most convenient way of observing the hair cycles is afforded by colored ani-

mals. Pigment production in the hair papilla declines before the hair attains its full length. Hence the proximal portion of the fully grown hair shaft is lighter in colored animals than the distal part. On the other hand, the newly formed hairs are strongly pigmented and shine through the epidermis even before their eruption above the skin surface. Areas of regrowth are therefore strongly pigmented. Growing black hairs impart to the skin a characteristic bluish-black color before their appearance on the skin surface. The dark areas of active growth can be easily differentiated from pale resting skin areas after the fur has been clipped or parted by blowing gently over it.

It has been established that the different mammalian species belong to one of the following three groups (122):

1. Animals in which the follicles are in a continuous state of activity, like the sheep or Angora rabbits (122); in the latter species, however, there may be resting periods (71).

2. Animals with more or less simultaneous cyclic activity in all follicles. In other words, hair is replaced all over the body at about the same time, and adjacent follicles are in the same phase of the hair cycle. These simultaneous phases of growth and inactivity are especially well pronounced during the first few hair cycles in life. As the animal grows older, the replacement of hair proceeds more and more in a wavelike fashion. With increasing age, the spread of these waves slows down. After several cycles the replacement of the hair becomes irregular, because a new wave may start before the expiration of an old one and thus several waves may spread simultaneously over the skin. The final pattern in these species is one of irregular island-like growth. In these islands follicular activity occurs simultaneously in neighboring follicles, while the adjacent resting skin areas contain dormant follicles only. In the next cycle the previously active islands become dormant, and the

surrounding resting areas burst into activity. To this group of mammals belong, among others, the mouse, (118, 488), rat (208, 111, 59), and rabbit (386).

3. In a few species with cyclic follicular activity, replacement of the hair is distributed in a diffuse or random way among the hair follicles (so-called "mosaic pattern"). In other words, individual hair follicles exhibit a great deal of independence, and, at a given time, two neighboring hair follicles may be in different phases of the cycle. The guinea pig (393) and cat (156) are representatives of this group. Man also belongs in this category. In man, however, the situation is further complicated by the fact that the length of the hair cycles varies in different body regions and that individual hair follicles show a great deal of autonomy in the lengths of their periods of growth and rest (101, 100).

B. MICROSCOPIC CHANGES IN THE HAIR SHAFT AND HAIR FOLLICLE DURING THE HAIR CYCLE

In all species studied, the main microscopic changes which the hair follicles and hair shaft undergo are essentially the same. Hair cycles have been most extensively studied in the mouse and the rat. In these species the early hair cycles have a duration of about 1 month; thus the changes are telescoped within a short period of time and may be followed with relative ease.

In the following section a brief outline will be presented of the main changes that the hair shafts and hair follicles of man or animals undergo in the course of the replacement of hair. As already mentioned, the hair cycle affects other cutaneous structures as well; these will be discussed later.

The hair cycle has been divided into three stages: (1) the *anagen* stage, the phase of active proliferation; (2) the *catagen* stage, a transitional phase, characterized by cessation of active proliferation and by club hair formation; and (3) the *telogen* (final) or resting stage (118). After this stage the hair cycle starts all over again.

The first stage of the hair cycle to be

discussed will be the catagen stage. In this phase the actively growing hair is transformed into the dead club hair. Ordinarily, the hair matrix differentiates into the internal root sheath and the hair shaft, with their respective component layers. This differentiation ceases in the catagen stage. The lowermost cells of the matrix degenerate, thereby discontinuing the production of the internal root sheath. At the same time, the connective-tissue papilla escapes from the grasp of the lower matrix cells and is no longer inclosed by the matrix. The matrix cells over the papilla, instead of producing organized keratin, begin to multiply to form a column of dedifferentiated epithelial cells. During this process the papilla becomes a free cluster of connective-tissue cells, similar to what it was in the primordial germ state.

The club itself is formed in the upper portion of the keratogenous zone, and initially it consists of a brush of incompletely keratinized cells. Later the hair club ascends in the hair follicle and becomes fully keratinized. This ascent is caused by the multiplication of the matrix cells, which produce an epithelial cord. This solid cellular cord reaches up to the level of the sebaceous gland. The club is firmly fixed in this column by keratinized spindle-shaped processes. Subsequently, because of cellular degeneration, the epithelial column becomes greatly reduced in size and shortens to a nipple-like protrusion under the epithelial sac in which the club is inserted.

The hair club finds its final resting position (telogen phase) in the upper portion of the hair follicle, at the level of the attachment of the arrector pili muscle. The final length of the visible hair shaft is reached at this time. All these events are illustrated schematically in Figures 1, 2, and 3.

Although the club hair is a dead structure, a foreign body inserted into the skin, it would be erroneous to assume that its attachment in the follicle is loose. The club forms a firm, hard anchor which keeps the hair shaft in the resting position. The removed club hair can be easily differentiated, even with the naked eye, from hair in the

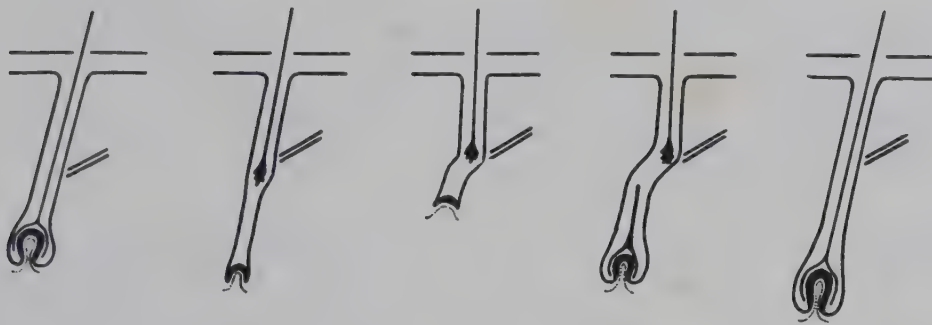


FIG. 1.—Coarse scheme of hair cycle. From Schwanitz (386). (Reproduced by permission of Springer-Verlag.)

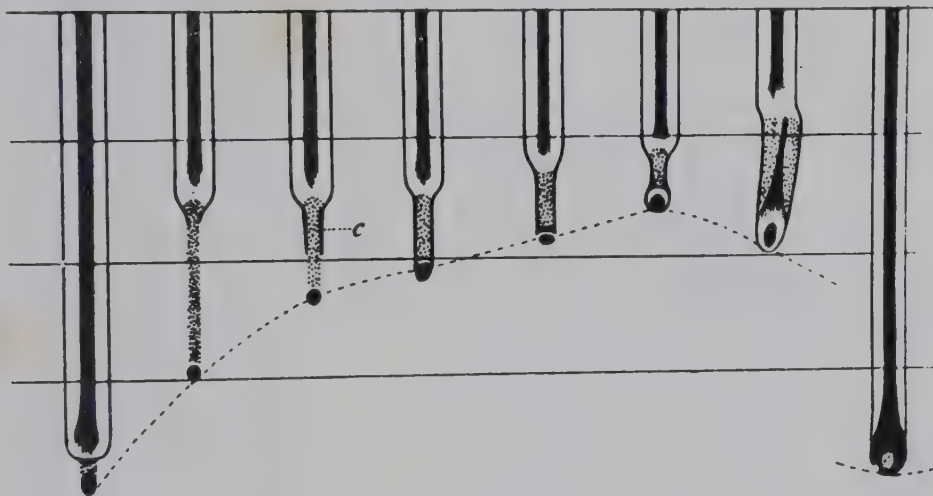


FIG. 2.—Schematic representation of hair cycle. From left to right: club formation and gradual ascension of hair papilla, with shortening of epithelial column. Second figure from right: formation of new hair. From Pinkus (350). (Reproduced by permission of Springer-Verlag.)



FIG. 3.—Half-schematic representation of hair cycle. *a*, beginning club formation; *b*, ascension of club to its resting position with attached epithelial column; *c*, *d*, *e*, and *f*, gradual ascension of papilla, with shortening of epithelial column; *g*, formation of new hair. From Pinkus (350). (Reproduced by permission of Springer-Verlag.)

anagen stage. The club hair has a solid, hard, dry white node at its proximal end; the white color of the club is due to lack of pigment. On the other hand, the root of the still growing hair is easily pliable and somewhat moist and sticky; in pigmented hairs it has the same color as the rest of the hair shaft (350). Under the microscope one can see the growing hair inserted in a sac of

epithelial cells; the lowermost portion of the hair shaft is somewhat expanded and pigmented.

In the anagen stage the first sign is increased mitotic activity in the nipple-like protrusion under the hair club. The strand extends downward and envelops the papilla; by continued cellular division the base of the entire follicle stretches downward. The



FIG. 4. Human club hair pulled out in vivo with epithelial sac attached. (Courtesy Dr. Albert M. Kligman.) $\times 27$.



FIG. 5.—Proximal portion of growing (anagen) human hair. Heavily pigmented root. Note attached root sheath extending 2–3 mm, in vivo above base. (Courtesy Dr. Albert M. Kligman.) $\times 19$.

first sign of the new hair is a thin keratinized dome; this dome differentiates into the hair shaft of the new hair, which pushes its way past the club hair. In man, as a rule, the club hair is not immediately extruded from the

follicle but remains there for a while, simultaneously with the new hair (Figs. 4-9). For a short time (in man, seldom exceeding a week), two hairs emerge from the same follicle: the old club hair and its successor



FIG. 6.—Earliest stage in human club hair formation. Club hair resting in upper part of follicle, with long cordlike epithelial connection to papilla. Note marked thickening of basement membrane and retraction of peripapillary portion of bulb from papilla. (Courtesy Dr. Albert M. Kligman.) $\times 39$.



FIG. 7.—More advanced stage in club hair formation. Shortening of epithelial cord, with ascension of papilla. (Courtesy Dr. Albert M. Kligman.) $\times 39$.



FIG. 8.—Most advanced stage of club hair formation. Epithelial column reduced to small nipple-like protrusion at lower end of follicle. (Courtesy Dr. Albert M. Kligman.) $\times 39$.



FIG. 9.—Regeneration of new hair. Papilla surrounded by incompletely differentiated hair bulb. Beginning development of keratogenous zone in new hair. (Courtesy Dr. Albert M. Kligman.) $\times 75$.

(448). In some species the club hairs remain in the follicle for several subsequent hair generations, which explains the occurrence of one kind of multiple hairs (352).

C. ARTIFICIAL INDUCTION OF THE HAIR CYCLE

The hair cycle is one of the most fundamental physiologic processes displayed by the skin. Nevertheless, its basic importance for dermatologic research has not always been fully appreciated. Many of our erroneous notions about hair growth, a number of preparations and methods introduced for the purpose of restoring hair, and a multitude of experimental studies of the skin are of little or no value because of the experimenters' failure to recognize the role of the hair cycle.

One of the most commonly encountered errors is the failure to realize that any non-specific cutaneous "irritation," short of damaging the hair follicles, may initiate a new hair cycle in animals. Such an irritative measure is: plucking of the hair in mice (104, 84, 85, 73, 74, 76-78), rats, hamsters (75), or rabbits (386). In mice it is sufficient to pull at the hair without actually removing it, in order to initiate a new hair cycle. In man, complete removal of a hair early in the cycle may lead to a shortened period of rest, followed by a new and usually normal cycle. There is no indication of a subsequent compensatory lengthening of the cycles. Breaking of the hair, even deep within the follicle, has no obvious effect on the human cycle (101). These findings have been utilized in the treatment of alopecia areata, when, at an early stage, the regenerating fuzz was completely removed, in an effort to induce the regrowth of a second, heavier hair generation in the plucked area (191).

Rubbing (massage) also initiates a new hair cycle in dormant hair follicles in rabbits (274, 127, 140), cats (156), and dogs (274). Failure to recognize this simple fact is directly responsible for the introduction into dermatologic therapy of such magic hair restorers as cholesterol and for unwar-

ranted speculations about its nutritive value for the hair follicle (226).

Cutaneous application of irritating substances, like tar, phenol, chrysarobin, croton oil (273), or cignolin (127) on the rabbit skin may induce new hair growth. Injection into rabbit skin of such divergent products as skatol, acetic acid (70, 339), staphylococci (220), mistletoe extract (246), or infection with smallpox or diphtheria (194) may have the same effect. Dormant follicles of underfed rats have been stimulated to activity by repeated cutaneous applications of benzoic acid, tincture of cantharides, or capsicum, xylene, acetic acid, or by injection of histamine (60). It has been claimed that the irritation must be severe and conducted for several days in order to be effective. It must be remembered, however, that in activations of resting hair follicles a certain time-lag period elapses before the effect becomes noticeable macroscopically. This latency period is 8-12 days long and represents the time required for the activated hair to reach the skin surface or to cause the characteristic deeply pigmented areas in colored animals. This period cannot be shortened in any way. It is understandable, therefore, why it was impossible to advance the beginning of the second hair cycle in the rat (which normally starts on the thirty-fourth day of life) when the irritating treatment was administered on the twenty-second day of life (60).

Among physical factors which may precipitate a new hair cycle in animals, the following have been reported: infrared light (70) and ultraviolet light in rabbits (127, 273, 339, 378), ultraviolet light in guinea pigs (278), or visible light in rabbits (47) and in ferrets (40). Light appears to be the physiologic stimulus of the hair cycle in certain species, as will be discussed later.

Among traumatic influences, the stimulating effect of freezing (378), of wounds (339, 470, 275), and of Thiersch transplants (42) on the hair follicles of the rabbit may be mentioned. Skin transplantation in hairless rats stimulates hair growth not only at the

site of the operation (104) but all over the body. The mechanism of this interesting phenomenon is obscure (166).

All these "irritating" agents have one feature in common: they all produce hyperemia. Increased vascularization of the skin and hair follicles is characteristic of periods of active hair growth (122) and may be observed even with the naked eye in the so-called "islands" of hair growth (248) in older animals. These islands are invariably thicker and more hyperemic than the surrounding dormant skin areas (248, 477). Nothing definite is known about the mechanism by which the increased blood flow activates the resting hair follicles.

While there is no doubt that it is possible to initiate growth in the resting hair follicles of animals with "irritating" measures, the problem of whether or not such an activation may be effected in human hair follicles has never been satisfactorily investigated. The random, mosaic-like distribution of resting and growing hair follicles in man and the physiologic variations in the lengths of the resting and growing periods of individual follicles make research in this field difficult. The problem is closely related to the question of influencing the *rate* of hair growth and is of great practical importance. Expressed in simple terms, the question is whether or not it is possible to induce or to accelerate hair growth in man by the application of "irritating" (hyperemia-producing) measures. A detailed discussion of this problem will be presented later.

D. CHANGES IN THE SKIN DURING THE HAIR CYCLE

As mentioned earlier, the hair cycle is not an isolated phenomenon, restricted to the hair follicles only, but affects many of the morphologic aspects, functions, and responses of the entire skin. For this reason it would be more appropriate to speak about a "skin cycle" rather than a hair cycle (337). This term would be justified in the case of animals with well-defined cyclic hair growth, like young rats and mice. Our knowledge in this field is still rather fragmentary; co-

ordination of the scattered information is attempted in this section.

The thickness of the cutaneous layers varies with the stages of the hair cycle. The epidermis of the mouse, which under "normal" conditions is 2 to 3 cell layers thick, becomes two to three times thicker in the early anagen phase, between the third and seventh day of the cycle. This thickening is the result of a burst of mitotic activity. As the hair shaft is produced, the epidermis returns to its "normal" thickness. At about this time, the end of the first week, the corium increases by about 50 per cent of its "resting" thickness, and the adipose layer thickens two to three times. The "resting" thin appearance of these layers does not become manifest again until the telogen stage is reached. The sebaceous glands expand during the growing stage and return to their smaller "resting" size in the telogen phase (75). Similar observations have also been made in the rat (122, 337, 165).

The water content of the rat skin is highest during the period in which the cells of the hair follicles divide. During the first 6 days of life, the water content amounts to about 80 per cent; after the sixth day it falls to 65–70 per cent and declines gradually to 60–65 per cent after the seventeenth day of life. At the beginning of the next cycle, around the twenty-eighth to thirtieth days of life, the water content rises again to nearly 70 per cent between the thirtieth and fortieth days, after which it falls once more (440). These results confirm earlier measurements of the tissue fluid content of rat skin, made by Butcher and Grokoest (63). However, the causal relationship between hair growth and increased tissue fluid content is not well understood. Thus adrenalectomy in the rat leads to increased hair growth without affecting significantly the water content of the skin (63).

The oxygen consumption of the skin of rats is lowest in the telogen phase (0.92 mm³/mg dry weight/hr), according to Butcher. The greatest oxygen uptake, a 30 per cent increase over the resting level, occurs on the thirty-first day, just prior to the

beginning of follicular activity. After this peak, oxygen consumption falls again to the low "resting" value (61). In all likelihood, cessation of hair formation is at least partly responsible for the precipitous drop in the oxygen consumption of the rat skin at the age of 7 days (25) to "resting" levels, which are in excellent agreement with those reported by Butcher.

A correlation was also found between the hair cycle and the fragility of the capillaries in the rat. The capillary fragility was greatest prior to the growth of hair and was least pronounced during the resting stage. Hormonal influence which promoted hair growth also increased the capillary fragility. It was postulated that hair growth in the rat was probably related to the condition of the capillary bed (253).

After their destruction with methylcholanthrene, the sebaceous glands regenerate in the anagen phase only. When hair growth is arrested, no new sebaceous glands differentiate until hair development is resumed (298).

Histochemical changes are prominent during the hair cycle. In the period of active proliferation the papilla and external root sheath contain a high concentration of alkaline phosphatase, while in the telogen phase there is practically no enzyme present (230, 231). Basophilic and alcohol-resistant material is demonstrable both in the hair papilla and in the cytoplasm of the follicular epithelium only during the anagen phase of the first and second coat of the rat. This material, presumably acid polysaccharides, is believed to be associated with periods of keratin formation (427). Similarly, the uptake of a basic dye, toluidine blue, is high during the growth phase and low during the rest phase in the skin of albino rats (21). These findings indicate a cyclic variation in the acid, negative groups of the tissues. The characteristic distribution of glycogen in actively proliferating human hair follicles (299) is not altered during rest periods (244).

The effect of carcinogenic compounds on the skin of mice is modified by the hair cycle. Observers agree that there may well

exist a cyclic fluctuation in the growth of chemically induced skin tumors. However, the exact nature of this fluctuation is controversial. Thus Mottram (305) stated that warts induced by carcinogens showed reduced growth and decreased size in the late anagen phase. In this stage the warts were pale because of reduced vascularity; the epidermis showed very few mitoses. The warts resumed their growth only after the hair had entered the resting stage. The theory was advanced that in the late anagen stage the growing hair diverted the blood from the papillomata and thus interfered temporarily with their proliferation (305). This theory may also account for the observation that mice which had a greater capacity to regenerate the hair which had been lost after the application of tar were more resistant to the carcinogenic effect of tar than animals with poor regenerative power (408). On the other hand, Jonkhoff claimed that, after application of tar to mice, papillomata appeared during periods of hair growth and sometimes disappeared during periods of rest (232). In rabbits, after painting with 9,10-dimethyl-1,2-benzanthracene, papillomata appeared in originally active sites of hair growth. The appearance of the tumors inhibited hair growth in other areas (477). Obviously, the relation of the hair cycle to epidermal carcinogenesis requires further investigation with standardized applications of potent carcinogens on pure strains of mice.

The question of whether or not the hair cycle has any influence on the sensitivity of the hair follicles to X-rays will be discussed under the alopecias (p. 634).

Growing hair is not disturbed by methylcholanthrene, while resting hair is avulsed. Wound healing is greatly accelerated in the presence of active hair follicles (75).

Thallium affects the growing hair only (p. 633). In rats poisoned with thallium, the thallium content of the skin parallels the growth of the first and second hairy coats. The highest concentrations of thallium were found in 8-9-day-old and 44-45-day-old animals. In the intervening dormant phase

the thallium content fell to less than half the peak values observed during periods of active growth (440).

In summary, it may be stated that skin containing anagen hair follicles reacts differently from skin with catagen or telogen follicles. "Anagen" skin is an actively proliferating tissue, responding somewhat like embryonic tissue, while telogen skin may be likened to more mature and, generally speaking, more resistant structures. Whether or not this analogy is satisfactory, it is certain that the skins of two animals of the same species or two skin areas in the same animal may exhibit different morphologic features and may respond in an entirely different manner to various physiological or pathological stimuli. Failure to recognize the fundamental role of the hair cycle in affecting the morphological and physiological aspects of the animal skin is one of the common sources of erroneous conclusions and contradictory findings reported in investigative dermatology. In order to avoid such pitfalls, it is advisable to observe certain precautions. Whenever possible, the experiment and its control should be conducted on symmetrical areas of the same animal, where, as a rule, hair follicles are in the same state of activity; or animals with mosaic pattern of hair growth, like the guinea pig or cat, should be used. Experiments carried out on mice or rats, using littermates as controls, are open to criticism, because individual variations in the time of appearance of hair cycles have been reported; the very first cycle may already be subjected to such variations (165).

E. THE PHYSIOLOGICAL CONTROLS OF THE HAIR CYCLE

The mechanism by which these profound changes during the skin cycle are brought about in animals has been the subject of numerous investigations. The cyclic alterations between growing and resting periods suggested that they were under cyclic hormonal control; it was believed that ovarian hormones were primarily responsible for the initiation of the various phases of the hair

cycle. This hypothesis was seemingly supported by the observation that in rabbits the regular loss of fur in spring and fall (194) paralleled the involution of the corpora lutea. These animals, as well as other species (40), use the shed hair to build nests for their newborn (443). A correlation between the ovarian and hair cycles has also been noted in the mouse (170), rat (59), and ferret (40). Pregnancy is responsible for a lengthening of the resting periods (105), a high percentage of resting hair follicles (106, 107), and occasional hair loss (106) in the guinea pig.

Nevertheless, it is not permissible to state that there is a direct causal relationship between the cyclic activities of the ovaries and the hair follicles. Castration or ovariectomy did not suppress the hair cycle in the rat (112, 59, 158), cat, dog, horse, or in cattle (59); and it has been postulated that both ovarian and follicular activities may be under the common control of a third factor, such as the pituitary gland. In some species this gland may have a prominent role in modifying the hair cycle. In ferrets, hypophysectomy suppressed the sexual and hair cycles. In this species the physiological stimulus for the pituitary may be the sunlight, because severing of the optic nerve has the same suppressive effect on the sexual and hair cycles as does extirpation of the pituitary (40).

In other species, however, there is no evidence that the pituitary is essential for the maintenance of the normal cyclic activity of the follicle. In rats, for example, hypophysectomy slightly prolongs, but does not abolish, the hair cycles (158, 159, 402). Similarly, the extirpation of other endocrine glands did not abolish the basic cyclic pattern of follicular activity (112).

At present, the best available evidence suggests that, while the cyclic activity of the hair follicles may be modified by hormones, *the factors bringing about the changes in the hair cycle are essentially inherent in the hair follicle itself*. This thesis was supported by Butcher when he transplanted rat skin under the skin or into the peritoneal cavity

of the same animal; as long as hair growth was maintained in the skin transplants, the cyclic activity of the follicles was preserved (62). It has also been shown that denervation in rats had no effect on the spread of the cyclic hair waves over the denervated areas. The original direction of the hair wave was

F. THE HUMAN HAIR CYCLE

In man, the life-cycles of hairs may be measured in two ways:

1. The simplest method consists of measuring the rate of growth of individual hairs and of calculating from the over-all hair length the life-span of the hair. This method

TABLE 3
HUMAN HAIR CYCLES

Region	Reference	Length of Cycle	Length of Growth Period	Length of Rest Period
Scalp.....	313	<i>Ca.</i> 16 months (males); over 20 months (females)	*	*
	350	5-7 years	*	*
	160	Av., 4 years	*	*
Cilia, eyebrows.....	350	150 days	30 days (+15 days catagen)	105 days
	160	112+28 days	*	*
Beard.....	347	7-11 months (possibly up to 3 years)	4-11 months	10-75 days
Dorsum of finger.....	101	72, 78, 125 days	*	*
Dorsum of hand.....	351		Summer av., 197 days; winter av., 166 days	141-83 days
Ulnar side of hand.....	51	Summer av., 170 days; winter av., 150 days	Av., 75 days	26-110 days
Axilla.....	313	202-4 days	*	*
Chest.....	349	First series: summer av., 140 days; winter av., 124 days; second series: summer av., 210 days; winter av., 189 days	*	Av., 70 days
Pubes	451		11-18 months	12-17 months
Perineum } in women.....			11-23 months	9-12 months
Lumbar }			4-7 months	9-12 months

* Not reported.

not changed by rotation of the skin transplant and was not stopped by an area epilated with X-rays (122).

Thus in the hair follicle we are confronted with an epidermal structure which bursts into periodic mitotic activity; this activity may be somewhat modified by hormones but cannot be stopped by any means, short of the actual destruction of the follicle.

has some inherent limitations and errors. It is a measure of the growth period only and fails to yield any information about the length of the resting period. Moreover, the tips of the hair shafts are commonly worn down or broken off, introducing a further error into the calculations. Nevertheless, on the basis of data gathered by simple growth-rate measurements, it is permissible to state

that longer hairs, as on the scalp and beard, have a longer life-span than the shorter hairs on other parts of the body; also the exceptionally long (over 1 yard) scalp hairs of some women may grow without interruption for decades.

2. The only accurate, though tedious and time-consuming, method is the direct observation of individual hairs after permanent identification of their follicles (India ink, tattooing, or relation to well-defined sites, like nevi, etc.). Experiments of this type have been carried out by several authors. Their findings are summarized in Table 3. An analysis of these data reveals the following conclusions.

Hairs on different parts of the body have cycles of various lengths. The longest cycles are characteristic of hairs on the scalp, where in exceptional cases only 3 or 4 hair generations may succeed one another throughout life (350). On other parts of the body, 2 cycles per year is a satisfactory average figure. This figure brings to mind the 2 yearly molting periods of most animals; indeed, Leeuwenhoek thought that every spring he could observe a seasonal molting on himself (101). However, objective observations by Pinkus failed to reveal any seasonal distribution of hair loss; rather, it appears that hair loss on all parts of the body is more or less uniformly distributed throughout the entire year (351).

Hairs growing next to one another may have cycles of different duration. Neighboring follicles may be in different phases of their cycles, one resting, the other growing (101). In sharp contrast to many animal species, under normal circumstances no regional pattern of simultaneous growth or

rest can be observed in man.

The ratio of growing to resting periods varies a great deal in different body regions. Hairs on the scalp have very short resting periods. The resting periods of hairs on the legs are longer than the periods of growth, while the reverse is true for axillary and pubic hair. The eyebrows, hair on the dorsum of the hands, and hair on the ears have resting periods one to one and a half times as long as growing periods (448, 450).

Hair grown during the winter months has a somewhat shorter life-span than hair produced in summer (351, 51, 349). In combination with the greater rate of growth during the summer months (p. 617), the net result is that "summer hair" grows to a greater length than "winter hair" from the same follicle. For hairs grown on the chest from the same follicle, Pinkus recorded an over-all length of 45 mm. during summer and 38 mm. during winter (349).

The life-span of individual hairs grown from the same follicle, although relatively constant, may show a great deal of variation. "Each follicle has a considerable degree of autonomy in its development and function, but its activity is in some measure regulated by factors external to itself" (448). A series of cycles from eight adjacent follicles and their variations have been strikingly illustrated by Danforth (101).

Between two succeeding hair generations there may or may not be hairless intervals. The new hairs may coexist with the old hairs for periods ranging from 1 to 8 weeks, although, in general, the old hair is pushed out shortly after the appearance of the new hair (448).

III. THE RATE OF HAIR GROWTH

The average rate of hair growth is 0.1–0.4 mm/day. This figure is influenced by a number of factors.

A. REGIONAL DIFFERENCES

The highest rates of growth have been observed in hairs of the scalp and chin.

Daily growth rates for scalp hair have been measured as 0.35 (313), 0.39 (447), 0.22–0.43 (162), 0.33–0.45 mm. in children and 0.27–0.40 mm. in adults (354); for the chin, 0.38 mm/day (313) and 0.21–0.31 mm/day (348). The axillary hair grows almost equally as fast, 0.30 mm/day (313), 0.42

mm/day (447). Daily growth rates on other parts of the body are lower: 0.20 (313) and 0.23 mm. (447) for the thigh; 0.22 mm. (447) for the arm; 0.16 mm. for the eyebrows (313).

TABLE 4

SEASONAL VARIATIONS OF DAILY GROWTH RATES OF BEARD (124)

Month	Mean Temperature	Measured Daily Growth (Mm.)
January.....	58	0.305
February.....	54	.386
March.....	61	.404
April.....	70	.458
May.....	74	.464
June.....	81	.516
July.....	83	.533
August.....	82	.538
September.....	79	.545
October.....	73	.533
November.....	64	.495
December.....	60	0.375

growth (162, 395). Daily temperatures apparently have no influence on the growth rate (395, 449). However, when, in careful studies, average monthly temperatures were correlated with average monthly growth rates, a distinct increase in hair growth during the summer months was noted (Table 4) (124).

D. EFFECT OF AGE

The growth of scalp hair is fastest between fifteen and thirty years of age and

TABLE 5

VARIATIONS IN GROWTH RATE OF HAIR WITH AGE (313)

	AGE (YEARS)		
	21-30	45-52	64-66
Axillary hair growth rate (mm/day).....	0.36	0.33	0.24
Beard growth rate (mm/day)	0.42	0.37	0.37

TABLE 6

RATES OF HAIR GROWTH ON THIGHS AT VARIOUS AGES

	AGE (YEARS)										
	9-11	13-14	17-20	19-22	21-25	21-30	20-30	30-40	40-45	45-52	64-66
Daily growth (mm.) (313)	0.13	0.16	0.25	0.19
Weekly growth (mm.) (448).....	1.42	1.45	1.45	1.56	1.57	1.62	1.85

B. SEXUAL DIFFERENCES

Scalp hair grows faster in women (0.34 and 0.36 mm/day) than in men (0.31 and 0.34 mm/day) (313). The reverse is true for axillary hair (0.31 and 0.33 in males; 0.29 and 0.30 in females) (313). These differences may result from the fact that hair growth in these areas is influenced by the gonads. Eyebrows grow at the same rate in both sexes (0.15-0.16 mm/day) (313).

C. SEASONAL VARIATIONS

Earlier authors maintained that the seasons do not influence the rate of hair

declines between fifty and sixty years (162, 464). Decrease in the growth rate of axillary hair and beard begins at an earlier age (Table 5) (313). Pubic hair retains its growth rate with aging (448). On the other hand, hair on the thigh increases its growth rate during the first five decades and declines thereafter (Table 6) (448, 313). Eyebrows grow at about the same rate throughout life (313).

E. DIURNAL VARIATIONS

Notwithstanding contrary claims of earlier authors (35), it appears that hair growth

is decreased at night, a phenomenon analogous to that observed in nails (161), probably as a result of decreased mitotic activity.

F. INFLUENCE OF THICKNESS OF HAIR SHAFT

Trotter claims that the average rate of growth per unit of time is roughly proportional to the diameter of the hair. If hairs selected in different regions were of the same diameter, the above-mentioned variations in growth rate would be considerably less (448). This observation requires confirmation with studies of growth rates on hairs with accurately determined diameters.

G. EFFECT OF CUTTING AND SHAVING

According to popular belief, cutting and shaving of the hair accelerates its growth. This belief has no basis in reality. All experimental investigators agree that cutting has absolutely no influence on the rate of hair growth (50, 98). The effect of shaving is somewhat more controversial. Common experience teaches us that men who shave daily are not inflicted with an ever increasing beard, and accurate daily measurements of beard growth objectively confirm the impression that shaving does not affect the growth of hair (162, 449, 50, 447). However, when measurements of the rate of beard growth were made at shorter intervals, it was revealed that, immediately following shaving, the hair grows at a more rapid rate. This increase is followed by a compensatory decrease within a few hours. The over-all effect of shaving in the course of a day is thus the same as that of cutting, i.e., none. The immediate increase of the growth rate for a few hours after shaving is a somewhat puzzling phenomenon; it is not due to the hyperemia caused by the shaving process, as "false shaving" has no such accelerating effect (395). It is conceivable that the process of shaving represents a strong mechanical stimulation which in itself is capable of causing an immediate increase in growth rate, followed by a compensatory decrease within 24 hours (161).

H. PHYSICAL FACTORS

The influence of physical factors on the rate of hair growth is of great practical importance. Innumerable abuses have been perpetrated in this field, and false advertisements exploit a misled public day after day. Even physicians have contributed to this deplorable situation by carrying out poorly controlled experiments.

The one circumstance which probably has given rise to more confusion than any other is the fact that animals react to all kinds of physical or chemical irritation with activation of their dormant hair follicles. This activation, in turn, leads to growth of new hair, as mentioned above. *No evidence has been presented that activation of resting hair follicles may be elicited in human subjects by the use of irritating measures.* All observations indicating the beneficial effect of physical factors in alopecia areata must be disregarded in view of the unpredictable course and frequently self-limited nature of this disease.

Although physical measures have not been shown to induce a new cycle in human hair, there are several observations proving that irritation, if sufficiently prolonged, increases the rate of hair growth in man. Development of hypertrichosis on chronically irritated areas is a well-known empirical fact. In a recent study Ressimann and Butterworth described the localized hypertrichosis on the forearms of psychotic patients who bit themselves again and again in these areas; the repeated trauma caused hyperemia and led to increased length and number of the hairs, which was attributed to the failure of the old hairs to shed. There was no indication of new formation of follicles (357). Longer and thicker hairs have been found on the shoulders of sack carriers from the intermittent pressure and rubbing of that site (92, 93). Localized hypertrichosis has also been observed around wounds treated with oxalic acid (109), in the vicinity of healed psoriatic (250) or neurodermatitic lesions (27), after solar dermatitis (27), following prolonged applications of hot baths (213), bandages (particularly with plaster

of Paris casts), tincture of iodine, mercurial ointment, around burns and scars (163), and in areas repeatedly irradiated with ultraviolet light (382). In all these instances the prolonged hyperemia was believed to be the provoking factor.

However, even in these well-documented cases the hypertrichosis was not present in all subjects, but only in a varying percentage of them. It is therefore justifiable to ask: How severe or prolonged must the irritation be before it causes increased hair growth in all subjects? This question has never been objectively studied. It has been simply assumed that any rubbing, brushing, ultraviolet irradiation—in brief, any measure which produced hyperemia—would be beneficial for hair growth. This assumption has not been supported by objective studies. While it is true that strong faradic or mechanical stimulation (161) or sunburn (447) causes an immediate increase in the growth rate of hair, this initial furthering effect is followed by a compensatory decrease within 24 hours. In the long run, hyperemia-pro-

ducing measures of short duration have no effect on the rate of hair growth. This thesis has been supported by Trotter, who found no stimulation of hair growth from petrolatum rubbed in lightly (447). No effect on the hair length could be observed from tincture of iodine (rubbed in twice weekly for a month), from Vlemminckx' solution or from ultraviolet irradiation, even though these measures caused erythema and (in the case of tincture of iodine) dermatitis with scaling (162). Scalp lotions had no greater effect on hair growth on one side of the head than did the solvents applied on the opposite side (136).

To summarize: prolonged hyperemia causes an increased rate of hair growth and thickening of the hair shaft in a certain number of people. Individual differences appear to play a major role. There is no evidence indicating that hyperemia of short duration has any lasting effect on hair growth. The exact dividing line between "short" and "prolonged" hyperemia has never been established and requires further study.

IV. CHANGES IN HAIR WITH AGE

With the exception of the soles, plantar surfaces and interspaces of toes, side surfaces of feet halfway up to the ankles, palms, palmar surfaces and interspaces of the fingers, glans penis and clitoris, the entire body of the fetus is covered with a fine silky coat of hair, called the "primary hair." As early as during the fourth to eighth months of fetal life, thicker hairs differentiate in certain areas, thus setting off the scalp hair, eyebrows, and eyelashes from the rest of the body hair.

Under normal conditions this primary hair is replaced during fetal life with the so-called "secondary hair" (292). In other words, the primary hair is shed, and a second generation of hair appears. The time at which this replacement takes place varies in different individuals. The exact time is controversial. According to Pinkus (350), the replacement of hair begins in the eighth or ninth month of pregnancy and may not be

completed until the sixth month after birth. There is almost general agreement, however, that there is only one generation change in fetal life.

In a rare hereditary anomaly, the replacement of the primary hair with the coarser hair of later life never takes place, and subsequent hair generations always retain the original form and distribution of the primary hair. Individuals thus affected are covered with a dense silky hair coat. For some unknown reason this retained fetal hair may reach considerable length and, when allowed to grow, covers the face and forehead. The short and barely visible lanugo hairs of normal people are replaced with long, fine hairs. The hairy forehead and face impart the appearance of a shaggy dog to the person thus affected; hence the popular expression "dog-man" to denote this condition (350, 292, 245) (Fig. 10). This anomaly is associated with severe defects in

dental development; the latter imperfection allows us to calculate the approximate age at which this developmental anomaly sets in. The disorder has been described under a number of names, the most appropriate being "hypertrichosis primaria" (292). Another common term, "hypertrichosis la-

(350). A similar situation prevails in the "Angora" races of animals. While the hereditary nature of this condition is established beyond doubt, a unique case of "acquired hypertrichosis lanuginosa" in a woman with metastatic carcinoma has also been described (279).



FIG. 10.—Hypertrichosis lanuginosa s. primaria in a family. From Pinkus (350). (Reproduced by permission of Springer-Verlag.)

nuginosa," is less apt, as not only the primary lanugo hair but all the primary hair is preserved in this condition. However, it is well to keep in mind that, although clinically resembling hypertrichosis, this disorder is essentially one of underdevelopment, or rather inhibited development, of the secondary hair and hence has also been called "hypotrichosis" or "trichostasis lanuginosa"

The secondary hair of children comprises, in addition to the fine lanugo hair of infancy, the so-called "vellus" (101), i.e., the coarser type of lanugo hair which gradually replaces the infantile lanugo on part of the extremities and some parts of the trunk before puberty and the coarse hair of the scalp, eyebrows, and eyelashes. In the early part of puberty the so-called "inter-

mediate hair" begins to develop (350), a very regular and beautifully distributed dark, silky hair. During and after puberty the terminal hair begins to appear, a process which may last well into adulthood. This terminal hair includes, in addition to the secondary hair of children, the pubic and axillary hair, the beard, hairs in the openings of the ear and nose, and varying amounts of coarse body hair.

Throughout these stages, morphologic changes take place in the hair. Such changes in scalp hair have been most thoroughly studied by Trotter and Duggins from birth to the fourteenth year of life (452, 119, 453, 120). These changes affect the hair index, the size, and the medullation of the hair.

The hair index is the figure obtained by dividing the least diameter of the cross-section of the hair by its greatest diameter and multiplying this number by 100. The more the cross-section of the hair approximates a round shape, the higher is the index; a low index denotes an ovoid cross-section.

During the first 2 years of life the index drops sharply, after which there is no regular trend observable. In other words, the shape of the scalp hair changes from a more nearly round to a more oval one during the first 2 years (452).

The area of a cross-section (thickness) of the hair increases rapidly and uniformly during the first 3 or 4 years of life, less rapidly and uniformly during the next 6 years; thereafter the trend is one of slow increase or of leveling off. In other words, the hair becomes coarser after birth. It appears that at least in some follicles each successive hair surpassed its predecessor in size during the period of observation which, in the work

of Trotter *et al.*, ended with the fourteenth year (452).

Medullation is extremely rare at birth and during the first 2 months of life. The incidence of medullated hairs thereafter rises until the end of the first year, when almost half the scalp hairs are medullated; thereafter medullation declines to 23 per cent at 2 years and is followed by a gradual rise to the end of the fifth year, after which no particular trend could be observed (119).

Of practical importance is the increasing coarseness of hair in most parts of the body until old age. This increase in the diameter of the hair proceeds regardless of external influences. The popular belief that shaving in women is responsible for the increasing diameter of hairs on the face or extremities has not been proved experimentally. The increasing coarseness is physiological, and there is no evidence that this process is accelerated by normal cosmetic procedures (486).

The observation that the hair thickens with progressing age has been indirectly supported by physical measurements which showed that the elasticity, tear resistance, and ability of the hair to be stretched increase with age into adulthood and decrease with old age. All these three properties are related to the thickness of the hair (281).

With increasing age the cystine content of the hair increases in man (432) and in animals (328, 41, 361, 272), although some authors were unable to detect such a rise in human hair (83). The detection of the optically anomalous *d*(-)-leucine in the hair of old horses would warrant the extension of this work to other species and other amino acids (472).

V. THE PROBLEM OF NERVOUS FACTORS

Although numerous workers have described the well-known nerve plexuses and nerve endings around the hair follicles of man (499, 238, 433, 173, 81, 229) and of animals (457, 326, 227), the belief that the hairs have special "trophic" nerves (258) must be regarded as obsolete. Changes in

the length or density of body hair after diseases of the nervous system (213, 364, 241, 484) are probably due to vasomotor disturbances; and there is no evidence for the existence of special nerve fibers, essential for the integrity and development of the hair follicles.

Evidence which proves that the hair follicles can differentiate without nervous control may be summarized as follows:

1. Hair can be grown in tissue cultures. With this technic the hair follicles of the mouse (200, 201), rat (310), and guinea-pig embryo (418) have been observed to develop hair shafts in vitro. The presence of the dermal papilla may be essential for the hair growth, as in their absence no organized hair is formed (200).

2. Homoeotransplants of hair follicles have been successfully achieved in human subjects (327). In Thiersch grafts, for many years after the operation, the type and density that characterized the hair at its original site are maintained in the area of transplantation (214, 493).

3. In man, sectioning of the sensory nerves or of the sympathetic supply (493) or complete denervation (116) has no effect on hair growth. Sympathectomy in animals may cause changes in hair growth, because of interference with the blood supply (212, 372, 373, 185), although Cannon and his co-workers found that sympathectomy had no effect whatsoever on the growth of hair in cats (66).

Nervous factors have been believed to

play an important etiologic role in two types of human alopecia, without being supported by acceptable evidence. These two conditions were alopecia from thallium, as discussed later (p. 633), and alopecia areata.

The belief in the nervous origin of alopecia areata apparently received strong support from Joseph's early experiments on sympathectomized cats. When the cervical ganglia were extirpated in this species, round patches of baldness appeared (234, 29). The hair loss was always temporary. Later, in carefully controlled studies, Aubrun showed that the alopecia was indirectly due to postoperative hyperesthesia in the denervated area which incited the animals to violent scratching; as a result, the hair on the head was rubbed off. When, after the operation, the heads were placed under protective caps, no alopecia developed (11-15). In studies on man, cutting of the sensory nerves, of the entire sympathetic supply of the scalp, or of the second cervical nerves had no effect on the growth of scalp hair (493).

In summary, it may therefore be stated that there is no evidence whatever that human hair growth is under direct nervous control.

VI. NUTRITIONAL FACTORS

In animals a great variety of nutritional deficiencies lead to impaired growth of hair or complete alopecia. A list of these factors is presented in Table 7. It is apparent that hair loss (including circumocular alopecia, called "spectacled eyes") is a common and nonspecific response in nutritional deficiencies. Although some of the vitamins, such as inositol (also called "mouse anti-alopecia factor"), have been singled out as of prime importance in the maintenance of the normal fur, at present the chain of events leading to impaired hair growth and the biochemical interrelations between the nutritional factors are so incompletely understood that we have no right to attribute specific hair growth-promoting powers to any of these factors (see chap. 27).

We are even less justified in transferring directly the results of experiments conducted in deficient animals to clinical cases of human baldness. Unless experimentally induced, virtually all human nutritional deficiencies are multiple deficiencies. However, it appears that, while hair loss in animals is an early and frequent sign of the deficiency syndrome, severe nutritional deprivations are necessary in man before hair growth is impaired. The reason for this discrepancy may well lie in the fact that in man a much smaller percentage of the entire protein metabolism is diverted into the formation of hair than in animals (291). Furthermore, there is no evidence that the commonly occurring human alopecias are in any way connected with nutritional deficiencies.

In spite of these differences in the responses of human and animal hair follicles to nutritional deficiencies, individual nutritional factors have been tried time after time in the treatment of human baldness, with generally disappointing results (465, 329).

Among the nutritional factors alleged to promote the growth of hair, a few gained wide popularity at one time or another. Three of these agents—the hair hydrolyzates, cholesterol, and pantothenic acid—will be discussed below.

Hydrolyzed hair as a promoter of hair growth was introduced by N. Zuntz after World War I. This therapy was based on the observation that, in animals kept on a diet deficient in proteins or cystine, the growth of hair was impaired and could be restored to normal by the addition of hair hydrolyzates to the diet. The hydrolyzed hair provided the organism with the building stones needed for the production of hair (500). Subsequently the use of hair hydrolyzates in the treatment of alopecias spread rapidly. Beneficial effects were claimed mostly in alopecias which were either reversible, such as postinfectious alopecias, or had an unpredictable course, as alopecia areata (344, 46).

On a theoretical basis, the use of hair hydrolyzates in the treatment of human alopecias is not justified. As has been pointed out by Rothman (369), these preparations have no advantage over any adequate protein which provides the body with essential amino acids. These proteins are broken down into individual amino acids, and the hair germ cells can build hair from these building stones as well as from hair hydrolyzates. Moreover, there is no evidence that the common alopecias in man are due to a deficiency in cystine or proteins. Under experimental conditions in rats, when the diet contained an adequate amount of cystine, further addition of this amino acid to the diet did not accelerate the rate of hair growth and had no definite effect on the composition of hair (401).

In summary, the still present use of hair

hydrolyzates (228) in any form is based on unscientific speculation and has no demonstrable value in the treatment of human alopecias.

Cholesterol has been advanced as a therapeutic agent in the treatment of alopecias on the basis of experiments performed in

TABLE 7

NUTRITIONAL DEFICIENCIES CAUSING IMPAIRED HAIR GROWTH IN ANIMALS

Deficient Factor	Species	Reference
Protein.....	Rat	204
Protein or cystine...	Rat	271
Cellulose (? hay)....	Guinea pig	306
Carbohydrate (α - or β -lactose as only source of carbohydrate).....	Rat	133
Lecithin.....	Pig	446
Fatty acid.....	Rat	355
Cystine.....	{ Rabbit	3
	{ Rat	1, 401
Methionine.....	Rat	207
Vitamin A.....	Rat	297
Riboflavin.....	Rat	422, 134 192, 216
Biotin.....	{ Mouse	319
	{ Rat	130, 317, 320, 407
	{ Pig	95
Inositol.....	{ Mouse	489-92, 282, 283
	{ Rat	407, 94, 318
Pantothenic acid...	{ Mouse	283, 346, 302
	{ Rat	186, 423
	{ Monkey	287
	{ Swine	487
Pyridoxine.....	Rat	9
Vitamin E.....	Rat	460
Chloride.....	Rat	330
Iodide.....	Pig	203
Iron.....	Rat	96
Copper.....	Rabbit	400
Zinc.....	{ Mouse	108, 322
	{ Rat	129, 152

rabbits. In this species, rubbing with ointments containing cholesterol (lanolin) promoted hair growth by activating the dormant follicles (226). As has been pointed out in a previous section (p. 611), any non-specific irritation, such as the mere act of rubbing the skin, induces a new hair cycle in rabbits. When adequately controlled experiments were carried out, percutaneous ap-

plication of cholesterol was not found to have any superior value over the inunction of any ointment (274). These objective rebuttals did not prevent the commercial promotion of cholesterol-containing hair lotions (235, 239). The use of egg-yolk shampoos and of other preparations which contain the "hair-food" cholesterol still enjoys some popularity (30).

Pantothenic acid and its derivatives, such as panthenol (pantothenic alcohol) are among the latest compounds used to promote hair growth. Good results have been claimed by a number of authors from local and systemic administration in the treatment of various types of alopecias (189, 236, 325, 256); but none of the clinical experiments was adequately controlled. An interesting observation concerns a hypothyroid child whose scant scalp hair could not be restored by thyroid medication, even though the other symptoms of hypothyroidism were alleviated. Administration of pantothenic acid brought about a dense growth of scalp hair (171).

Pantothenic acid is excreted through the hair; however, no difference was found in the amounts excreted in the hair of normal persons and of those with common baldness or alopecia areata (323). Urinary excretion

of pantothenic acid was also the same in normal individuals and in subjects with gray hair or with baldness (383). Thus there is no objective evidence available to support the assumption that pantothenic acid has a biochemical role in the production of hair. Final judgment on its clinical value as a hair growth-promoter must await controlled clinical studies.

Among the serious experimental studies on human hair loss from nutritional deficiencies, those of Goldman and his co-workers deserve mention. All the patients were children. In one series the hair loss was associated with other signs of deficiency syndromes (79); in another group the etiology either was uncertain or was attributed to faulty intestinal absorption due to intestinal parasites or to the administration of sulfonamides (175). Objective proof of the impaired hair formation was provided by the demonstration of changes in the physical properties of the hair (176). Administration of pantothenic acid was curative in many cases, although a definite relation between the intake of the vitamin and the improved hair growth could not be established. Impaired growth of beard in adult males on a starvation diet will be discussed elsewhere (p. 629).

VII. HORMONAL INFLUENCES

The endocrine glands affect hair growth in animals and in man. These hormonal effects, however, may manifest themselves in different ways in different species. For instance, in rodents the local (475) or systemic (476) administration of adrenal cortical hormones arrests the growth of hair and may even lead to follicular atrophy. In other groups of mammals and in man this effect cannot be demonstrated (20, 178). On the contrary, in man an excess of adrenal cortical hormones may lead to excessive hair growth in specified areas.

These discrepancies do not invalidate the results obtained in general from animal experimentation in the field of hair growth. Numerous agents, such as thallium, X-rays,

excessive doses of vitamin A, etc., have the same effects in animals and in man (p. 632).

The different responses of the pilary system to endocrine substances in man and in animals may be due to anatomical and physiological differences, such as the thin epidermis, large number and density of the follicles, and cyclic hair waves in common laboratory animals and differences in hormonal secretions (58). Furthermore, in furred species the formation of hair constitutes a much larger portion of the entire protein metabolism than it does in man (291).

Because of these species differences, it is not always easy to correlate experimental hormonal effects on animals with observa-

tions made on man. In this section the influence of hormonal factors on human hair growth will be discussed exclusively.

A. CLASSIFICATION OF HAIR FROM THE ENDOCRINE POINT OF VIEW

From the point of view of endocrine influences, human hair may be divided into the following groups, a classification modified from the ones originally proposed by Danforth (101) and Garn (164).

1. Hair that is the same in both sexes and is not primarily under hormonal control. This includes lanugo hair all over the body, eyebrows, eyelashes, and probably some of the hair on the extremities.

2. Ambosexual hair under endocrine control. This includes the coarse hair that is present after puberty to the same extent in normal representatives of both sexes. Ambosexual hair is represented by axillary hair and a major (mostly the lower) portion of the pubic hair; scalp hair may also be discussed in this category.

3. Hair that serves as a true secondary male character. Terminal hair on the face, beard and mustache, are the main representatives of hair which is present in all sexually mature and normal males³ but not in females. In most males varying amounts of upper pubic hair on and around the linea alba also belong to this group, as well as hair in the external ear canal, a distinct male characteristic (197). Finally, the larger amounts of body hair, like hair on the chest, shoulders, abdomen, and back of hairy men, are also sexual characteristics.

It has been pointed out that differences in the hairiness of males and females are quantitative rather than qualitative. There is not a single area which is hairy in men that may not become hypertrichotic in women under pathological conditions (28).

B. ADRENAL CORTEX

The promoting effect of adrenal cortical hormones on hair growth is illustrated by

3. The present discussion deals with the Caucasian race, as other races, such as some orientals, are characterized by scant or absent beard or body hair.

the hirsutism which develops in cases of hypercorticism in the female. Hypercorticism may develop from diffuse adrenal cortical hyperplasia, from benign or malignant tumors of the adrenal cortex, or from pituitary hyperfunction (368, 387, 321, 195, 151, 396). These types of hirsutism are caused principally by the secretion of androgenic hormones by the diseased adrenal glands. After removal of the hyperfunctioning adrenal tissue, the excessive hair falls out or subsequent generations of hair become gradually thinner. Many months may elapse after correction of hypercorticism before the hirsutism subsides or coarse hair is replaced by thinner hair. As a rule, hypertrichosis is the last sign to disappear, long after all other signs of hypercorticism have subsided.

Therapeutic administration of cortisone may cause hypertrichosis in the female. Hypertrichosis during and after pregnancy has also been attributed to hypercorticism (416).

In the female normal adrenocortical function is essential for the normal growth of pubic and axillary hair (p. 628).

Local application of adrenal cortical extracts has no effect on hair growth in normal individuals (178).

C. PITUITARY

The anterior lobe of the pituitary influences hair growth primarily by virtue of its adrenocorticotrophic hormone. Prolonged administration of adrenocorticotrophic hormone to female patients frequently causes hypertrichosis on face, trunk, and extremities. Pathological hyperpituitarism (Cushing's syndrome) also causes hypertrichosis of the face, chest, abdomen, and legs.

Hypopituitarism impairs the growth of hair. In Simmonds' disease the hair is thin and dry (213, 255); hair loss in the pubic and axillary areas is common (426). Similarly, lack of pubic and axillary hair is characteristic of pituitary dwarfs (5). Persistence of fetal lanugo was attributed to pituitary deficiency, because, according to Kylin, im-

plantation of the gland had a curative effect (255).

Hypertrichosis of the extremities and trunk occurs in acromegaly (131, 86), presumably an effect of the growth hormone.

D. MALE SEX HORMONE

Testicular hormone is responsible for the growth of the beard and, to a large extent, for the development of coarse hair on trunk and extremities in the male. Its presence is required also for the complete development of axillary and pubic hair in the male. In eunuchoids and prepubertal castrates the male type of hair fails to develop.

On the scalp, paradoxically, the male sex hormone creates conditions which may lead to early male baldness in genetically predisposed individuals. Eunuchs do not develop baldness but may do so when treated with testosterone propionate, provided that they have the hereditary predisposition (196). There appears to be a positive correlation between common male baldness and hairiness in other body areas (202).

The popular belief that hairiness of the trunk, with or without baldness of the scalp, indicates "virility"⁴ is obviously based on observations of absence of body hair in hypogonadal males as contrasted with the hairiness of normal males. However, the degree of hairiness depends not only on the presence of male sex hormone but also on hereditary factors. There is no correlation between the extent of hairiness and sexual potency. The terminal body hair is an indicator of the presence—in the present or in the past—of male sex hormone, but certainly not a quantitative measure.

All hormones with androgenic activity, regardless of their point of origin, whether from testicles, adrenal cortex, or ovaries, have a stimulating effect on the germinative epithelium of the follicles. In the case of testosterone propionate this stimulating

effect has been shown to be due to a direct local action on the follicles (4, 221, 474).

E. OVARIAN HORMONES

The ovaries may influence hair growth in a number of ways:

1. Albright hypothesized that production of estrogens stimulates the pituitary gland, which, in turn, stimulates the adrenal cortex to produce androgens (p. 625). The development of pubic and axillary hair in the female was thought to be due to the effect of these androgens (5). Actually, in females who developed Addison's disease before puberty, there is absence of pubic and axillary hair (242, 312). In primary ovarian insufficiency (5) and in ovarian agenesis (426) pubic and axillary hair does not develop fully because of insufficient stimulation of the adrenal cortex during puberty. This situation is remedied by the administration of estrogenic hormone. In adult life the ovaries are no longer required for this stimulation, and the loss of the ovaries no longer influences the growth and distribution of this so-called "sexual hair."

2. In cases of extreme feminization in man, the estrogens suppress the production of testicular hormone and may counteract the stimulating effect of male sex hormones (p. 626). However, hairiness does not depend simply on the outcome of a hypothetical battle between androgens and estrogens. No convincing evidence has been presented to indicate that local application or systemic administration of estrogens in clinically acceptable dosages has any effect on hair growth. Therapeutic attempts of this nature either have been disappointing or have led to questionable results (345, 138, 366).

3. The theca cells of the ovaries produce an androgenic hormone which has masculinizing effects. When produced in excessive amounts, this hormone may cause hirsutism. The malignant growth of theca cells, called "arrhenoblastoma," is the most extreme example of hirsutism of ovarian origin (419). Other pathological states of the ovaries may also cause hirsutism, such as the Stein-Leventhal syndrome (411, 410, 265).

4. The colloquial term for infantryman in French is *poilu*, "the hairy one" (64, 301). The Latin proverb: "Vir pilosus seu fortis, seu libidinosus," means "The hairy man is either strong or sexy" (257).

thecoma and hyperthecosis (157, 218), hirsutism of ovarian origin (417), or folliculoma (233). Luteomas and adrenal rest tumors also belong here.

In some of these cases, but not in arrhenoblastomas, the urinary 17-ketosteroid level is high, especially the fractions which are identical with the metabolites of testosterone (417, 219, 218).

F. THYROID

Normal functioning of the thyroid is essential for the adequate growth of hair. The gland may act indirectly by influencing other endocrine glands (particularly the gonads) or by its direct effects on tissues.

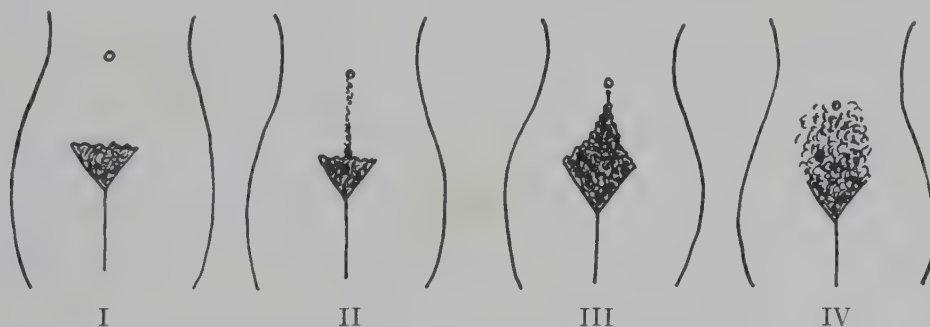


FIG. 11.—Four basic patterns of pubic hair distribution. *I*, horizontal; *II*, sagittal; *III*, acuminate; *IV*, disperse. From Dupertuis, Atkinson, and Elftman (121). (Reproduced by permission of Dr. Dupertuis and Human Biology.)

Diminished output of the thyroid affects the development of hair in intrauterine life. Persistence of fetal lanugo was observed in hypothyroid children (311). In such cases administration of thyroid caused new formation of hair follicles, which extended deeper into the corium than the follicles present prior to therapy (10).

In myxedema of adults the hair is scant, dry, and brittle (377, 414, 415). The hair loss has no particular distribution, although frontal alopecia (7) with greasy scales (300) and disappearance of the outer third of the eyebrows (277) seem relatively common. The latter (so-called "Hertoghe") sign has even been considered pathognomic for myxedema, but it occurs in other types of hair loss (e.g., in syphilis or thallium poisoning) as well (86, 316).

In hyperthyroidism, loss of hair on the

scalp, pubes, or the axillae may occur (86, 377). Hair loss on the scalp has sometimes the features of alopecia areata or totalis; possibly there is an underlying impairment in the function of the pituitary gland (482).

G. PUBIC HAIR

The classical distinction between "masculine" and "feminine" distribution of pubic hair was based on the observation that in normal men the pubic hair extends into the region in and around the linea alba, while in women its upper border is horizontal. More recently Dupertuis *et al.* (121) described four types of pubic hair distribution; the four patterns are characterized by their

upper borders and are as follows: (I) horizontal; (II) sagittal, the hair showing a linear extension in the mid-line over the linea alba; (III) acuminate, with a triangular upward extension pointing toward the umbilicus; and (IV) disperse, with the hair distributed over the abdomen without forming any discrete geometrical pattern (121) (Fig. 11). The frequencies of the various patterns in the two sexes at various ages are illustrated in Table 8, which shows that, with increasing age, men tend to develop a more hairy pattern. The disperse pubic hair is an absolute male characteristic; it never occurs in normal women.

From the point of view of hormonal influences, the areas covered with pubic hair may be divided into two sections: (1) a lower triangle with its apex pointing toward the symphysis and with a horizontal upper

margin; (2) the area above this horizontal border in which hair covers to a varying extent the linea alba and the surrounding abdominal skin. In some rare instances the two areas may be covered with hair of different colors, the upper area being darker (356, 39, 117).

Several observations indicate that the lower triangle may be under adrenal cortical control: (1) In Addison's disease of women there is frequent loss of hair in this region, provided that the deeper (probably the

growth of pubic hair. An illustration of this point was offered by Greenblatt. He reported the case of a woman with pituitary deficiency and amenorrhea. After the administration of estradiol, the patient's menstruation was restored, but she still did not grow any pubic hair. It was not until the patient received injections of testosterone that her pubic hair began to grow (179).

In men the upper portion of the pubic hair on and around the linea alba is under the influence of male sex hormones. As mentioned above, postpubertal castration abolishes hair in this region. In most normal women this area is not hairy. If androgen secretion is increased in females, hair growth may occur on and around the linea alba. According to Pedersen, menstrual anomalies, amenorrhea, and increased androgen excretion are twice as frequent in hypertrichotic women with hair in the linea alba as in hypertrichotic women without hair in this region. The extent and frequency of hairiness in other regions could not be correlated with the increased urinary androgen excretion and menstruation anomalies in hairy women (341, 342). This correlation between hair on the linea alba and increased 17-ketosteroid excretion in hypertrichotic women, found by Pedersen, could not be confirmed in a later work, and the problem appears to merit further study (340). After the menopause, the pubic hair becomes thinner, and if there has been a sagittal border it disappears (28). These changes were attributed to decreased adrenal cortical activity, as evidenced by a low 17-ketosteroid excretion in the urine (5).

Pregnancy has no effect on the distribution of pubic hair.

H. AXILLARY*HAIR

Axillary hair is controlled by the same hormonal factors as pubic hair. For this reason, as a rule, changes in the growth of hair from hormonal dysfunctions are similar in these two areas.

The growth of axillary hair in females requires both estrogenic and adrenal cortical secretion (5). In males there is no evidence

TABLE 8*
FREQUENCY OF PUBIC HAIR DISTRIBUTION
PATTERNS IN NORMAL MEN AND
WOMEN (121) (PERCENTAGES)

AGE (YEARS)	HORI- ZONTAL	SAGITTAL	ACUMI- NATE	DIS- PERSE
Males				
18.....	38.3	12.1	49.6	0
30-40.....	16.7	2.1	52.0	29.2
Females				
18.....	89.1	1.7	9.2	0
31-40.....	92.5	0	7.5	0

* Total number of subjects: 1,060 males; 309 females.

fasciculate and reticulate) zones of the cortex are affected (426, 5, 242, 39, 312). There is no quantitative correlation between the extent of hair loss and the level of urinary 17-ketosteroids (426). (2) There are four kinds of abnormal endocrine states in which low 17-ketosteroid levels in the urine are associated with loss of pubic hair, namely, Addison's disease (242, 312), Simmonds' disease, hypothyroidism, and senescence (5).

In men there is an obvious effect of the gonads on the growth of pubic hair. Postpubertal castration in the male reduces pubic hair in amount and to the level observed in normal females with a horizontal upper border (242). In women the estrogenic hormones alone cannot develop a full

that adrenal secretion is required for physiological growth of axillary hair. In males the peak of axillary hair growth is reached in the third decade; thereafter, it declines in a regular, steady curve (198).

In women the estrogenic hormones have no direct effect on axillary hair. It has been Albright's hypothesis (5) that estrogens promote growth of axillary hair indirectly by stimulating the anterior pituitary to secrete luteinizing hormone, which, in turn, enhances the secretion of an adrenal androgen. This view has been challenged by the observation that the scanty axillary hair of female Addisonian patients has regrown under therapy with cortisone, which is not an androgen.⁵

Axillary hair reaches its maximum development during the second or third decade and rapidly decreases after the menopause (28, 198). Eventually the hair disappears entirely in old age.

Decreased axillary hair production has been noted in conditions associated with decreased function of the testes. Thus in prepubertal castrates no axillary hair develops, and in postpubertal castrates the axillary hair decreases, though it does not entirely disappear. The weight of axillary hair apparently parallels the urinary values of 17-ketosteroids and androgens (198). In hepatic cirrhosis, axillary hair is frequently lost in both sexes, presumably because of the increased estrogen level. In men this loss occurs together with decreased growth of beard and gynecomastia (224, 308). Increased estrogen level may inhibit hair growth by suppressing testicular hormones.

It is of great interest that testosterone has a direct, local stimulating action on axillary and pubic hair. This effect has been observed in males with diminished gonadal function. When one axilla or one side of the pubic area was rubbed with alcoholic testosterone propionate, the hair became much more abundant there than on the control side, which was treated with alcohol alone

(4, 221). A similar effect has been noted also in abdominal areas (474).

Axillary hair is lost in the same abnormal endocrine states as pubic hair, namely, in Addison's disease of female patients (provided that the adrenal damage is sufficiently extensive) (426, 5, 242, 39, 312), Simmonds' disease, hypothyroidism, and extreme old age, all characterized by low urinary 17-ketosteroid excretion (5). An interesting observation concerns the loss of axillary hair in about half the patients with thyrotoxicosis, even before the appearance of other clinical signs of the disease (482).

An alleged sexual dimorphism has been described in the distribution of axillary hair. In males the axillary hair is supposedly distributed mostly along the medial side of the upper arm, in females mostly over the chest wall (132).

I. BEARD

Beard is a typical male character, dependent on the presence of male sex hormones. Eunuchs, eunuchoids, and other hypogonadal men have no beards. Decreased growth of beard in old age may be stimulated by administration of testosterone (80). Retarded hair growth, thinning, and greatly decreased growth of the beard in men living on a starvation diet has been attributed to decreased functioning of the gonads (225). However, these changes may equally well be attributed to the lack of an adequate supply of proteins needed for the production of hair. Estrogens, when given in large doses to males, decrease the amount of hair on the chin and cheeks (237). The beard is diminished in myxedema but is not affected in Addison's disease.

In women the growth of varying amounts of facial hair is an almost physiological manifestation after the menopause. It has been attributed to a temporary rise in adrenal cortical secretion, an explanation which is admittedly far from satisfactory. Under pathological conditions, growth of beard in women may be part of generalized hypertrichosis, due to adrenal virilism (pseudohermaphroditism), Cushing's syn-

5. Personal communication of Dr. Richard Landau.

drome, to the so-called Achard-Thiers syndrome ("diabetes of bearded women") (396), to virilism from gonadal dysfunction, or, as in the majority of cases, it may occur without any demonstrable endocrine disturbance (so-called "idiopathic hirsutism").

J. SCALP HAIR

Scalp hair is unique from a hormonal standpoint. While male sex hormones promote the growth of hair on other parts of the body, on the scalp these hormones may create conditions which lead to loss of hair. The presence of male sex hormones or of an excess of androgenic hormones of the adrenal cortex is essential for the appearance of alopecia frontalis (p. 631). Male sex hormones are required for the development of common male baldness. Aristotle has stated that eunuchs, children, and women do not get bald (374). Male-type baldness, however, can be elicited by the administration of testosterone in genetically predisposed eunuchs (196). It does occur in children affected by precocious puberty and in women suffering from virilizing disorders, deriving from excessive androgen secretion of the adrenal cortex or from excessive male hormone production in the ovaries (arrhenoblastoma) (394, 89, 22, 137, 284, 205, 343, 115, 485, 376). Simultaneously with luxuriant hair growth on the body, there may be partial or complete loss of scalp hair. It has been claimed that menopause may elicit male-type baldness (28), but such baldness is rare in women who do not suffer from endocrine disorders far beyond the physiological events of menopause.

The anomalous behavior of scalp hair in its response to androgenic principles has remained unexplained. Different theories have been advanced to explain the particular local conditions prevailing in the scalp which may give rise to the loss of hair in males. The main theories are as follows:

1. The scalp is a unique terrain, because the skin is stretched tightly over the skull. In puberty the rapid growth of the skull would place the overlying skin under in-

creased pressure and would interfere with the blood circulation. The decreased blood supply would cause more or less extensive damage to the hair follicles and would eventually lead to baldness (380). This theory and other similar views (428) do not account for the sudden development of male-type baldness in some virilizing disorders of women or for the hair loss occurring in adult eunuchs after treatment with male sex hormones. Increased tenseness of the skin of the scalp in bald people has been described (466, 467, 270); however, this decreased mobility of the skin may well be a secondary effect due to atrophy of the subcutaneous adipose tissue (270). Diminution of the blood supply to the hair follicles causes loss of hair both in man (314) and in experimental animals (498). However, the excitability of the cutaneous capillaries is also under the direct influence of male sex hormones (359), and there is no reason to postulate increased tenseness of the skin as the only possible mechanism which may interfere with the circulation of the scalp.

2. A possible local change brought about by male sex hormones may be an altered sebaceous secretion. Sabouraud assumed that there was a causal relationship between seborrhea and hair loss: seborrhea would create a favorable terrain for certain microorganisms which damage the hair follicles (374). This theory, now obsolete, has been superseded by the working theory that sebum may interfere with hair growth by virtue of its unsaturated components which cause hair loss in animals (141, 143, 144). A great deal more evidence is needed before the causal relationship between altered sebaceous secretion and hair loss is proved.

Whatever conditions are created on the scalp by male sex hormones, leading eventually to baldness, it is well to remember that hair follicles on the scalp are generally the most sensitive of all hair follicles to damaging effects. Such unrelated agents as thallium, X-rays, heparin, the dimers of chloroprene, or excessive doses of vitamin A, all attack most easily the hair on the scalp. It is obvious that conditions on the

scalp are different from those on other parts of the body. The difference may lie in a less adequate blood supply to the follicles or in

the long life-cycles of the scalp hair. In any case the problem is still unsolved and awaits further intensive study.

VIII. PATHOPHYSIOLOGY OF ALOPECIAS

Alopecia⁶ (calvities, baldness) or lack of hair is the result of a number of conditions in which the two following requirements are fulfilled: (1) hair growth is temporarily or permanently arrested; (2) the old hair falls out and is not replaced by new hair, temporarily or permanently.

A. PHYSIOLOGICAL ALOPECIAS

1. *Alopecia in the Newborn and Infant*

The fetus is covered with a dense coat of lanugo hair. Between the sixth and eighth months of pregnancy this hair disappears and is replaced by the so-called "secondary hair" of the newborn. Soon after birth, most infants lose varying amounts of hair on their scalps. The hair loss may begin as early as the first day after birth. It is most pronounced in the frontal and occipital regions; in the latter area, it is due mainly to rubbing. Within a few weeks the hair loss may spread over the entire scalp. Both sexes are equally affected. The condition is always reversible, and regeneration sets in within the first year of life (413, 54, 199). The same process has been observed in anthropoid apes (413).

The cause of this alopecia is unknown. The pattern of the hair loss has a marked resemblance to that occurring in adolescence and to common male baldness. For this reason the same theories have been advanced to explain all three conditions. One of these theories assumes that the rapid growth of the skull places the skin of the scalp under tension; the blood circulation to the hair follicles is impaired, and the hair stops growing (413). It is difficult to see how

this theory would explain the early occurrence and rapid spread of the alopecia. Increased sebaceous secretion in the newborn has also been implicated (262); whether or not "seborrhea" has any etiologic role in the development of any kind of baldness still awaits further proof (p. 630). It is probable that hair loss in many of these infants is caused by a delayed replacement of fetal hair; normally, this change takes place in intrauterine life.

The similar frontal distribution of the bald pattern in all these conditions does not necessarily signify identical etiologic factors, because hair follicles in this region are most susceptible to the inhibitory effect of a variety of agents which interfere with hair growth.

2. *Hair Loss of Adolescent Boys*

Before adolescence, children have a circular straight hairline which is similar in its outlines to a skull cap. During puberty, males lose hair in the frontal region in two triangular areas on both sides of the midline. This condition, also referred to as "calvities frontalis adolescentium," gives rise to the hairline resembling the shape of the letter M, which is present in the large majority of adult males. Between the ages of seventeen and twenty, one-third or more of all men already have these frontal incisures of the hairline (54).

The development of this hair pattern requires the presence of male sex hormones. It does not occur in eunuchs; they retain the straight hairline of the child throughout life (196, 199, 412). When treated with male sex hormones, eunuchs lose their hair in the frontal region; later the alopecia may progress toward the type of common baldness if there is hereditary predisposition (196). In precocious puberty the M-shaped hairline may develop at an early age (412). Women may also have a "masculine" hairline (54,

6. The word "alopecia" has often puzzled the etymologists. While there is general agreement that the word is derived from *alopex*, the Greek word for "fox," the connection of this animal with baldness has been obscure. One explanation has been that the grass withers and bare spots appear in the woods wherever the fox urinates (125).

199). The presence of this hairline in women does not in itself mean increased "masculinity" or "deficient femininity."

Frontal alopecia may be the forerunner of common baldness. Progression of the hair loss in the frontal region and merging with the gradually enlarging bald spot on the vertex leads to the pattern of common baldness, with only a fringe of hair left around the circumference of the scalp. This pattern of early male baldness requires the presence of male sex hormones (196).

3. *Peroneal Alopecia*

About every third man after the twentieth year of life loses hair on the lateral side of the lower leg above the ankle (199, 365). The skin in this area becomes gradually shiny and smooth, with only a few scattered hairs left.

The cause of this condition is unknown. Whether or not rubbing of the clothing plays any etiologic role remains to be established. In spite of previous beliefs (333, 445), it is without any pathological significance (365). The influence of hormonal factors is questionable; although peroneal alopecia has been observed in hypertrichotic women, it occurs in normal women as well. There is no correlation between the occurrence of common male baldness and peroneal alopecia (199).

B. TEMPORARY ALOPECIA FROM CHEMICAL AGENTS

Although temporary alopecia from chemical agents is not strictly within the scope of the physiology of hair growth, its discussion may be justified at this point. Chemical agents which interfere with hair growth may be considered as inhibitors of various biochemical processes which are necessary for the elaboration of normal hair. If we gain some insight into the nature of these various inhibitions, we may obtain some information about the mechanism of hair growth in general.

Chemical agents which have been described as causing loss of hair in man, by

interfering with hair growth and not by any keratolytic action, belong in one of the following categories:

1. A great variety of chemical compounds, used in industry or as therapeutic agents, may cause loss of hair by a non-specific toxic action. In most of these cases hair loss is only a sign of a general toxic reaction. In many cases the toxic hair loss is accompanied by a general disturbance in keratin formation, such as exfoliative dermatitis, or by anomalies of nail formation. It is beyond the scope of this chapter to enumerate all the substances which have been reported to cause loss of hair either because they were given in toxic doses or because of individual hypersensitivity to therapeutic doses. Among others, heavy metals (36, 31), arsenicals, potassium sulfo-cyanate (217), tetraethyl lead (434), atabrine (379), quinine, and ethyl carbamate (34) have been reported to have such effect.

2. Of much greater interest are chemicals which regularly cause loss of hair in the majority of human subjects. In these instances the alopecia need not be accompanied or correlated with other manifest toxic signs or symptoms. In spite of previous beliefs to the contrary, all these agents have a direct toxic effect on the follicular epithelium and thus interfere with the formation of normal hair. As mentioned earlier, the main point of attack in man is the hair of the scalp, the growth of which is arrested by all these agents. Interference with the formation of hair in some animal species is also a common property of all these agents. As a rule, hair loss in both man and animals is reversible. These compounds may be designated as direct, specific, or primary depilatory agents. Although their mode of action is not fully understood, it is probable that some of them inhibit mitosis (anticarcinogenic compounds, heparin) (332), while others prevent the normal process of keratinization, without interfering with cell division (thallium, unsaturated lipid-soluble compounds). The primary depilatory agents include the following chemicals: thallium, unsaturated lipid-soluble com-

pounds, substituted amino acids, folic acid antagonists and nitrogen mustard, and heparin and heparinoids.

1. *Thallium*

In spite of many publications on the subject (240, 177, 88, 309, 211, 57, 52) and much progress made in recent years, the exact mechanism of the depilatory action of thallium is still an unsolved problem. The view that the effect of thallium on hair growth was caused by disturbances of the sympathetic nervous system (56) and of the endocrine glands (55) was based solely on indirect evidence and could not be substantiated. Indeed, evidence was presented that there was no need to assume an indirect mechanism of action and that the depilation was the result of a direct toxic effect of thallium upon the hair follicles. It was found that thyroidectomized or ovariectomized animals lost their hair in the same way as normal animals (87, 72); that thallium exerted its depilatory effect on transplanted and presumably not innervated skin areas (72); and that in thallium poisoning degenerative changes occur in the follicular epithelium (454-56, 139).

Final proof of the "elective folliculotropic" effect of thallium came from Königsbauer and Thyresson. Königsbauer showed that depilation in animals could be obtained only when the hairs were in the growing stage. Severing of the sciatic nerve or spinal cord or removal of endocrine glands did not prevent the alopecia (247). In an exhaustive series of experiments (438-42), Thyresson found that the accumulation of thallium in the skin was greatest during periods of active hair growth. Studies with the radioactive isotope Tl^{204} revealed that thallium in the skin was largely taken up by the hair follicles (440). In the skin of newborn rats, thallium caused characteristic changes in the hair follicles, with vacuolization of the cells; this process was followed by a disturbed keratinization. The hair shaft gradually lost its distinct structure and turned into an amorphous mass; about 3 days after poisoning, degenerative changes appeared in

the hair bulb, hair growth was arrested, and finally the hair follicles completely degenerated. In tissue cultures of rat skin, thallium caused complete degeneration of the epidermis, with impaired keratinization. The experiments indicated that thallium did not disturb mitotic cell division but exerted a toxic effect upon the epidermal cells which formed keratin.

The biochemical nature of this toxic effect is unknown. The most probable mechanism is that thallium interferes with either the synthesis or the utilization of cystine. The administration of cystine and, to a lesser extent, of methionine protects rats against the toxic effects and alopecia caused by thallium (438, 187). On the other hand, sulfhydryl compounds, like BAL, have no such effect (44). This observation is in agreement with the finding that thallium does not combine with sulfhydryl compounds in vitro (146).

In summary, thallium disrupts the cellular differentiation which leads to keratin formation. The metal probably attacks an advanced link in this process, possibly by interfering with the incorporation of cystine into the keratin molecule.

2. *Unsaturated Lipid-soluble Compounds*

Reversible hair loss among workers in the neoprene rubber industry has been traced to exposure to intermediary products formed in the manufacturing process (388, 362, 324). These substances—the cyclic dimers of chloroprene—caused reversible baldness when painted on the skin of animals. The depilatory action was attributed to the presence of unsaturated double bonds ($-C=C-$) in the molecules. Other unsaturated compounds had a similar inhibitory effect on hair formation in animals (141-44, 147-49). Among these compounds, vitamin A is of special interest. When taken by mouth in excessive, nonphysiologic amounts, vitamin A caused loss of hair in over half the subjects suffering from hypervitaminosis A (425, 315, 358).

The exact mode of action of these compounds is unknown. It is believed that the

depilation is due to inhibition of free sulfhydryl groups of epidermal proteins. Hydrogenation of some of the unsaturated depilatory compounds, by saturating the double bonds, eliminated the sulfhydryl inhibition together with the depilatory action (143). In large amounts, *in vitro* vitamin A and its esters also inactivated sulfhydryl groups to a lesser extent than did other unsaturated compounds (145). A similar reaction has been postulated to explain the process of dark adaptation (468).

Human sebum also had a depilatory effect on animals. The inhibition of hair growth was due to the unsaturated fractions (both saponifiable and unsaponifiable) of sebum. Among the individual unsaturated components of sebum, oleic and linoleic acids and squalene have been shown to interfere with hair formation in animals (141, 143, 144).

3. Substituted Amino Acids

Leucenol (leucenin, mimosine), an alanine attached to a pyridine nucleus (2, 243), causes hair loss in human beings and in animals (251, 252). The compound is isolated from the seeds of an acacia-like shrub, *Leucaena glauca* (331). The mode of action of this toxic substance is unknown. It is conceivable that leucenol interferes with keratinization by displacing the natural amino acid alanine through competitive inhibition. In this respect it is noteworthy that two other substituted alanine derivatives have a depilatory effect in animals: abrin (methyl-tryptophane) in rabbits (461, 348) and dibromotyrosine or tetrabromotyronine in young rats (167-69, 335, 336).

4. Folic Acid Antagonists and Nitrogen Mustards

After administration of aminopterin (4-aminopteroylglutamic acid) and Amin-An-Fol (aminopteroylaspartic acid) the scalp hair is shed in a large number of patients. The effect is always temporary. Apparently, the follicular cells develop resistance to this agent, because the hair loss does not recur when the administration of the drug is con-

tinued (190, 135). Hair loss may also be elicited in mice; loss of whiskers occurs in the females of this species. It has been postulated that the depilatory effect was due to inhibition of ribonucleic acid synthesis (135).

Temporary alopecia has been observed after intra-arterial injection of nitrogen mustard in the follicles supplied by the injected artery (424). This is another example of the "radiomimetic" effect of this drug. Because of the great affinity of nitrogen mustard for a large number of reactive groups in the protein molecule, it is difficult to speculate about the mode of action of these compounds. However, it is interesting to note that both the folic acid antagonists and nitrogen mustard are antineoplastic agents. Interference with cellular proliferation may be caused by these agents in the rapidly dividing cells of the hair follicles.

5. Heparin and Heparinoids

One to four months after intensive therapy with heparin, the scalp hair falls out in over half the patients treated. Hair loss is most pronounced on the temples and in the frontal region; the alopecia is reversible. Similar observations have been made after the use of related anticoagulants, like Liquämin (294) and Thrombocid (285, 286). The mode of action of these compounds is unknown; the hair loss may be due to inhibition of mitosis (332).

C. ALOPECIA FROM IONIZING RADIATION

Reversible epilation from ionizing radiation affects the growing hair only. The influence of the hair cycle on the radiosensitivity of the hair follicle has been strikingly illustrated in an ingenious experiment by Gricoureff (181). When capillary tubes containing radon were implanted in rabbits' tongues, reversible epilation occurred on the cheeks of the animals. When the radioactive material was introduced in May or June, depilation took place 6 months later; insertion of the tubes in April resulted in hair loss within 3 weeks. According to Gricoureff, the explanation of this phenomenon was that

adult rabbits often have two yearly moltings—one in April, the other in November or December. The radiation attacked the hair follicles in their growing stages but did not affect the fully grown hair (181). In mice the actively growing hair could be epilated with low doses (300 r) of X-rays, while hair which was in the process of completing or had completed its growth (catagen or telogen stage) could be epilated with high doses (1,000 r) only. On the other hand, the *skin* of the mouse is less extensively damaged by

According to Geary, permanent X-ray epilation differs from temporary epilation mainly in the fact that in permanent epilation the higher doses of ionizing radiation injure the more resistant dermal papilla. Temporary epilation damages the more radiosensitive (epithelial) elements only, without permanently damaging the dermal papilla. Both the dermal and the follicular-epithelial elements are essential for the integrity and proper functioning of the hair follicle. In fact, the dermal papilla deter-

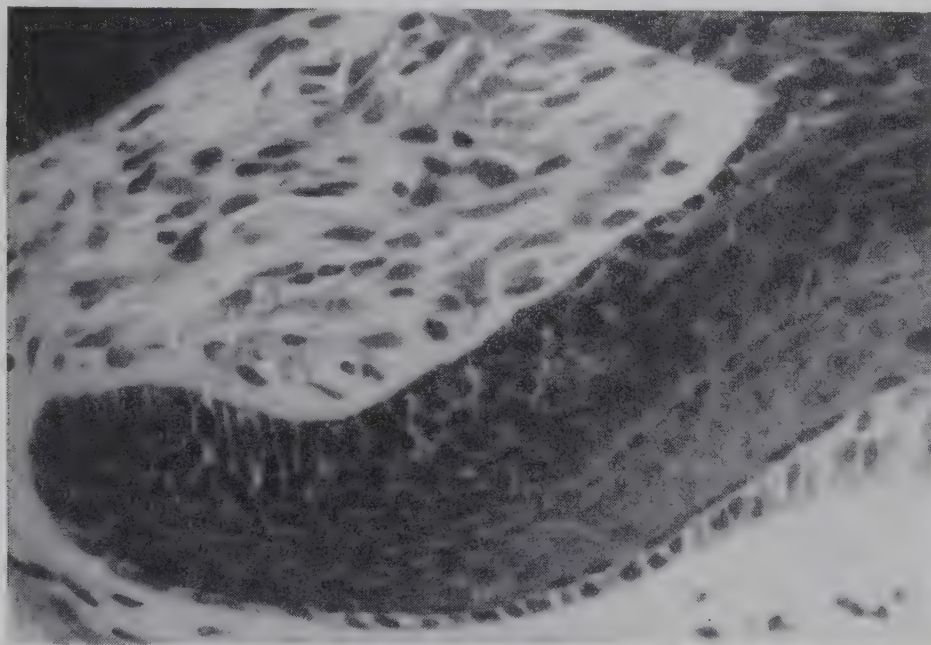


FIG. 12.—Normal hair bulb and papilla. (Courtesy Dr. Albert M. Kligman.) $\times 420$

X-rays or by methylcholanthrene when the follicles are in the anagen stage than in the telogen phase (76).

The most detailed study of this question has been carried out by Geary in albino rats (165). In this species the median effective dose of radiation which produced complete local epilation in half the animals exposed was found to be 1,720–50 r during the resting phase. Maximum radiosensitivity (hair loss after application of 1,030 r) occurred during the active phase of the hair cycle. Regrowth of hair was more rapid when the irradiation was carried out during the resting phase than when the exposure took place during the active stage.

mines the type of keratinous structure that the follicle is going to produce. This guiding influence of the dermis is one of the most fascinating and far-reaching concepts in skin physiology, well substantiated both in birds and in mammals.⁷ Its implications for skin grafting and for studies of epidermal and hair growth have not yet been fully realized and investigated.

A detailed study of the histological changes brought about by ionizing radiation in human follicles has been made by Kligman (244). His material is illustrated in Figures 12–15. These changes take place principally in the rapidly dividing matrix

7. For pertinent literature see Geary (165).

cells. The normal growth pattern of the hair is interrupted, and the hair enters a resting stage through a series of changes which are not unlike those which occur during the formation of club hairs in a normal hair cycle. Following radiation, the cyclic changes are greatly accelerated. Within a few days after irradiation, the cells of the lowermost

portion of the matrix degenerate, a process which partially frees the papilla. The internal root sheath produced by this particular portion of the matrix can no longer be formed. The matrix cells over the upper portion of the papilla, which normally form the hair cortex, undergo a partial degeneration. These cells no longer show the orderly and

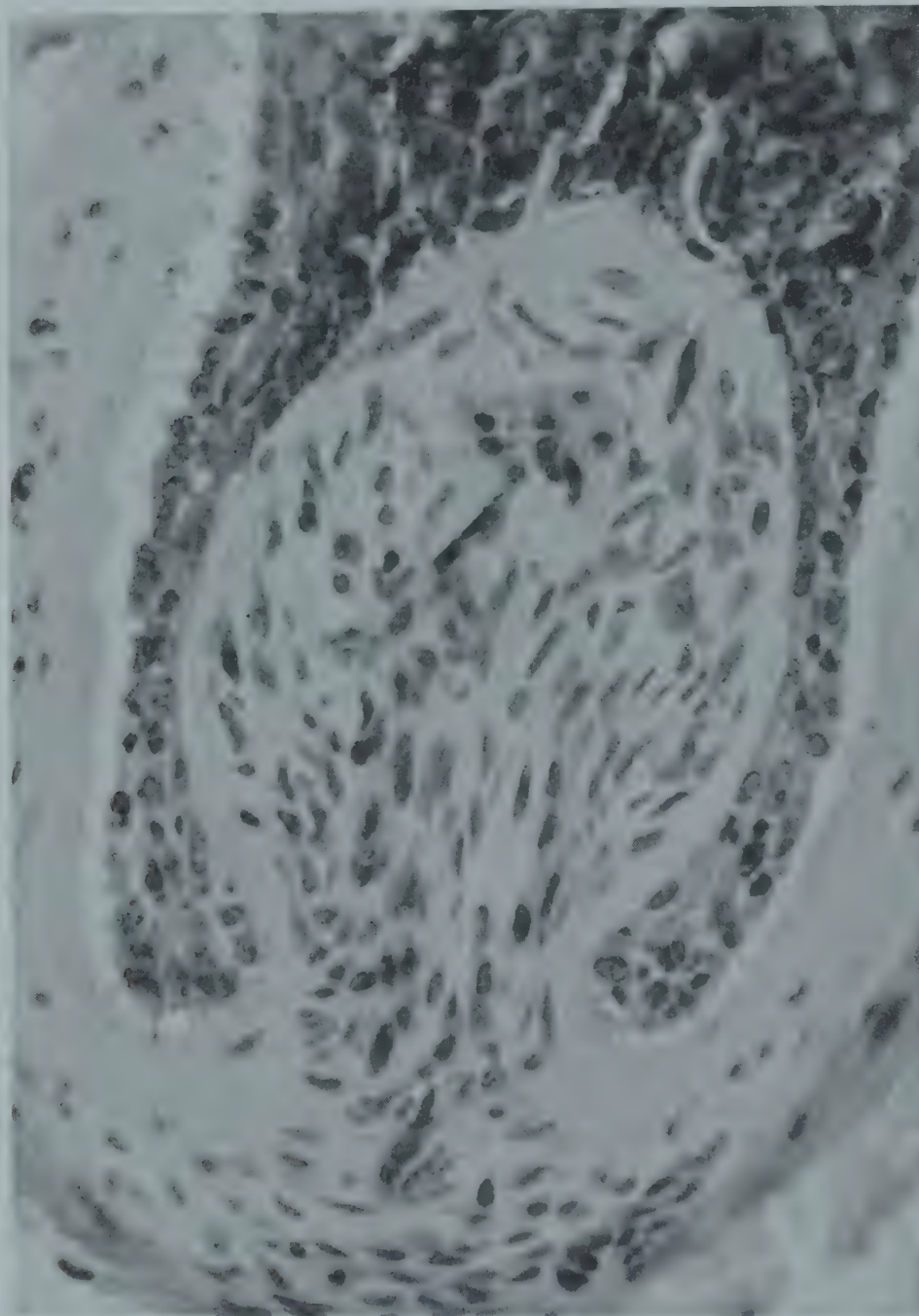


FIG. 13.—Thinning and degeneration of peripapillary portion of human hair bulb, 3 weeks after 300 r of X-irradiation. Absence of differentiation into component layers of hair. Glassy membrane markedly thickened, and papilla partially extruded at lower end. (Courtesy Dr. Albert M. Kligman.) $\times 580$.

gradual conversion into keratin through the keratogenous zone, but, instead, keratin production is abruptly inhibited. The surviving matrix cells no longer differentiate into keratin but proliferate to form an epithelial column, which gradually pushes the

arrested hair upward through the follicle.

Hairs arrested by radiation exhibit an attenuated tip. This pointed tip and a constriction of the hair shaft are clear indications of a gradual cessation of normal hair formation. These features were observed as

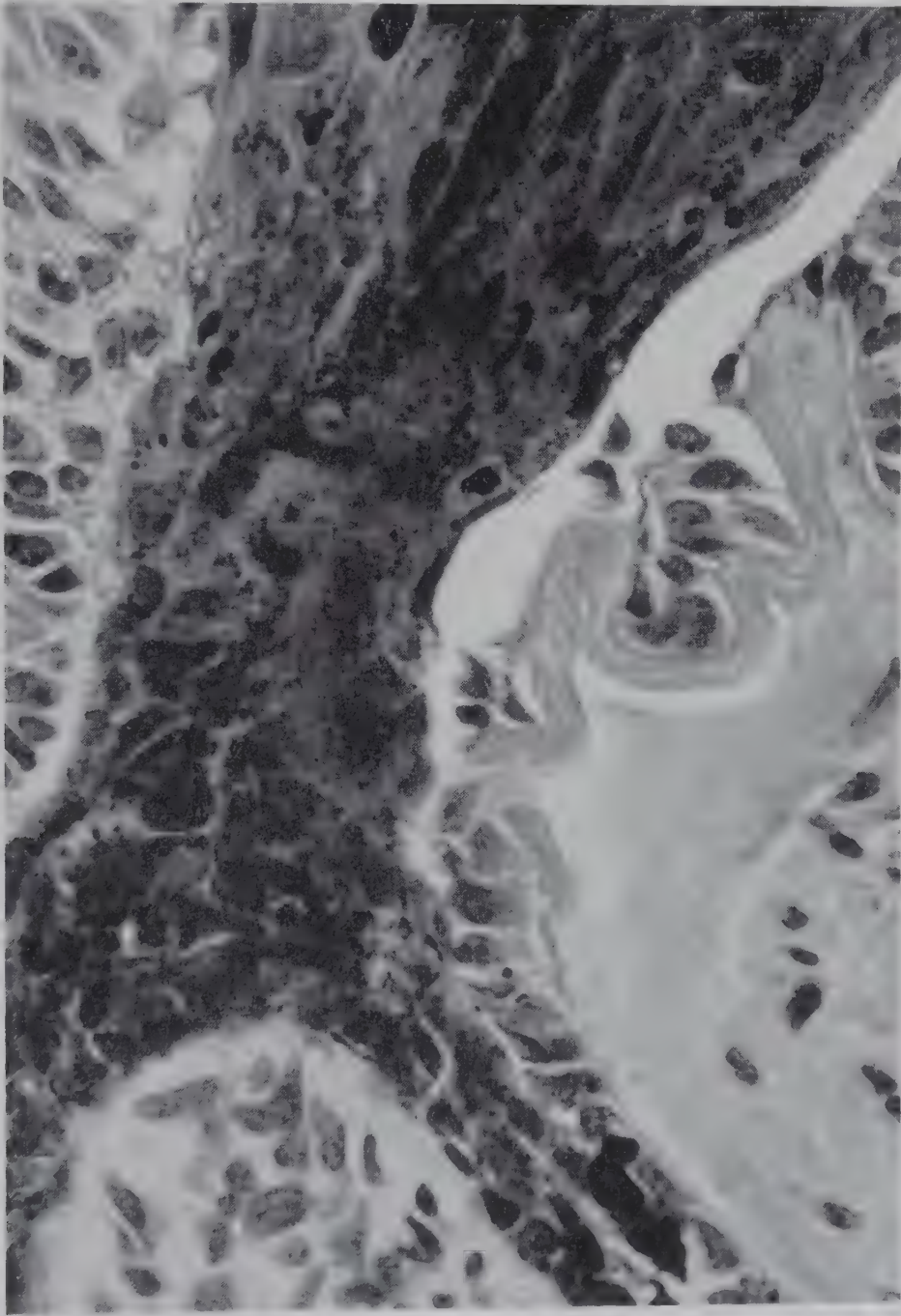


FIG. 14.— Degenerative cellular changes in suprapapillary portion of human hair bulb, leading to necklike constriction 10 days after 400 r of X irradiation. Note marked clumping of pigment and irregularities in size and shape of matrix cells; lack of differentiation into characteristic layers; and marked thickening of basement membrane, with folding and buckling, leading to sequestration of portions of the external sheath. (Courtesy Dr. Albert M. Kligman.) $\times 580$.

early as 1906 (481). The affected hairs are attached to the epithelial column by imperfectly keratinized spindles. Such hairs, unlike club hairs, fall out very easily. The papilla for the most part is no longer inclosed by the hair matrix cells and lies below the epithelial column. This epithelial column, composed of dedifferentiated epithelial cells, is in all respects similar to the column seen in normal club formation, except that it is longer. New hair formation also occurs in

of other regions. This greater susceptibility of scalp hair was noted also in its responsiveness to thallium, excess vitamin A, chloroprene dimers, and heparinoids. A satisfactory explanation for this regional difference has not been given in either case.

While the histological findings point with certainty to a temporary interference with normal keratinization, the exact nature of this inhibition is unknown. It may be similar to the metabolic disturbance caused by



FIG. 15. Avulsed human hair, 3 weeks after 300 r X-irradiation. Gradual attenuation of hair root, indicating progressive extinction of keratinizing activity of hair bulb. Absence of root sheaths. (Courtesy Dr. Albert M. Kligman.) $\times 39$.

the same way as in the normal cycle. Following the application of a temporary epilating dose of 350–1,000 r to the scalp, hair formation is arrested for 2–2½ months. Essentially, X-rays act like an antikeratinizing agent. Similar histologic changes have also been described in casualties of atomic bombs (268).

As in animals, in man the actively growing hair appears to be more sensitive to X-rays. After a dose of X-rays which causes temporary epilation, the club hairs are not affected but remain after the anagen hairs have fallen out (458).

Scalp hair is more sensitive to the epilating effect of ionizing radiation than is hair

thallium, because, according to some observers, there is a summation effect between the depilatory actions of thallium and X-rays (8), although this point is controversial (444).

The most probable mechanism by which X-rays affect hair growth is by inactivation of sulfhydryl compounds in the matrix of the follicles. It is known that ionizing radiations accomplish such effects by decomposing water into electrically charged ions. These ions, in turn, oxidize the free sulfhydryl groups of proteins and inhibit many of the enzymes which require the presence of free sulfhydryl groups for their activity. A sufficient amount of tissue oxygen is needed

for this inactivation; this is the reason why anoxic animals are relatively radio-resistant (24, 338). A similar resistance of anoxic human hair follicles was noted as early as 1909 by Schwarz in a simple experiment (389). Two wooden blocks were placed on the scalp, one loosely, the other under pressure. Both areas were then exposed to the same amount of radiation passing through the blocks. While the area which had its normal blood and oxygen supply underwent temporary depilation, the hairs in the compressed region were not affected. The protective effect of local anoxia against the depilatory effect of X-rays has also been demonstrated in mice (244).

The theory that X-rays cause reversible epilation by inactivating the free sulfhydryl groups of follicular proteins received strong experimental support from Forssberg's studies on guinea pigs. Reversible depilation in this species could be prevented if, immediately prior to the irradiation, cysteine was injected intracutaneously. The protective effect of cysteine was restricted to the injected site. Control areas injected with saline lost their hair (155).

These findings indicate that radiation damage to the hair matrix may be caused not only by direct absorption of the rays in the hair follicle and papilla but also by the indirect effect of a diffusible substance formed in the irradiated tissues. The formation of such a substance after irradiation was demonstrated by Van Dyke and Huff. When two rats were parabiotically united and one of them was irradiated, while the other was shielded, both animals lost their hair. Indeed, the hair loss was more marked, though of shorter duration, in the protected animal than in its exposed mate (459).

Incidental observations of various types of hair loss were made on victims of atomic bomb explosions (269, 268) and after severe exposure in an atomic accident (209).

D. POSTINFECTIOUS ALOPECIAS

Reversible diffuse hair loss may occur after infectious diseases, provided that the disease was accompanied by fever. The con-

dition is not specific for any particular disease. It may follow a number of unrelated diseases, such as erysipelas, pneumonia, typhoid, and influenza, to mention only a few (375). Postinfluenzal alopecia may reach epidemic proportions, as it did in the 1918 epidemic.

The hair loss is most pronounced in the anterior and parietal regions of the scalp; the temples may also be involved. It may reach extreme proportions. The time at which maximum hair loss occurs is somewhat controversial. Sabouraud stated that, on the average, $2\frac{1}{2}$ months (60–85 days) were necessary for the dead hair to fall out. Hence one could easily ascertain the date at which the febrile attack occurred by counting back about 10 weeks from the onset of the alopecia (375). Some authors believe that the latent period can be much shorter than 10 weeks (206, 501, 304, 266, 267); there is almost universal agreement, however, that an 8–12 weeks' latent period is most common (435). However, Sabouraud's contention that 10 weeks were needed for a club hair to fall out was fallacious. In the absence of histologic studies, we can only surmise the sequence of pathologic events. Direct microscopic evidence of the avulsed hairs is available, the most thorough and painstaking studies having been made by F. Pinkus (348). From these observations the following conclusions may be drawn.

The disease process interferes with the formation of normal hair; however, in the same way as after X-ray epilation, hair growth, though in an impaired form, continues for some time. Thinning of the hair root is a common feature (501, 266, 348). Normal club formation is apparently missing. The thinned and weakened hair is eventually pushed out from the hair follicle, but the resting time of such a weakened hair is probably different from the length of a resting stage during a normal hair cycle.

The weakening of the hair in postinfluenzal alopecia has also been ascertained with physical measurements (280).

After infectious diseases, the following additional microscopic changes occur regu-

larly in the hair: (1) true thinning of the hair without actual hair loss, which is most pronounced in the fully grown hair; (2) lighter color of the hair shaft, due to a temporary arrest of pigment formation and probable accumulation of air in the medulla; and (3) in medullated hairs, discontinuity of the medulla. All these signs are pathognomic of past diseases (348).

Whatever the underlying cause, hair loss after infections has almost always a favorable prognosis. Regrowth of hair starts soon after the onset of the alopecia, although it may take a long time before the hair reaches its former length and density. Exceptions, with scanty, poor regrowth, were seen mainly after typhoid fever.

Following a latency period of about 90 days, temporary alopecia may occur after apparently normal deliveries. The course of the alopecia is like that of the postinfectious type, and the underlying mechanism may be similar.

IX. PATHOPHYSIOLOGY OF ESSENTIAL (BENIGN, IDIOPATHIC) HYPERTRICHOSIS IN WOMEN

The great majority of women afflicted with superfluous hair growth have no demonstrable hormonal dysfunction. Therefore, this type of hypertrichosis has been called "idiopathic," "essential," or "benign." Attempts to demonstrate consistent changes in the androgen-estrogen metabolism in such cases have failed. In women with facial hypertrichosis who had hair growth on the linea alba, Pedersen found high average values of urinary 17-ketosteroids; his values on normal and hairy women, however, show considerable overlap (341, 342). Moreover, his data could not be confirmed (340). The alleged increase in the urinary androgen/estrogen ratio of hypertrichotic women is also debatable because of the overlap of normal and pathological values (172). At present there is general agreement that urinary 17-ketosteroid values are normal or subnormal in women with essential hypertrichosis (38, 65, 39).

Nevertheless, the failure to find con-

E. POSSIBLE HORMONAL FACTORS IN ALOPECIA AREATA

Alopecia areata, a disease of unknown origin, occurs in otherwise healthy persons with no demonstrable endocrine disorders. It seems, however, that its onset and course may be influenced by hormonal factors (469). Long before the advent of ACTH and cortisone, it was reported that administration of anterior pituitary extracts or implantation of the pituitary gland had a temporary curative effect in alopecia areata, alopecia totalis, and alopecia universalis (33, 437, 97). Recently, cortisone and ACTH have been shown to be effective in restoring the growth of hair in this disease (113, 114, 483). The beneficial effect may be based on restoration of a normal process of keratin formation in all keratinizing appendages, because concomitant disturbances of nail production are also improved. The effect is not permanent. The alopecia recurs if treatment is discontinued, unless there is a spontaneous remission of the disease.

sistent hormonal abnormalities in "idiopathic" hypertrichosis may well be due to insufficient refinement of the technics available for the study of adrenal cortical function. When the excretion of 17-ketosteroids in normal and hypertrichotic women was followed over longer periods, it was found that under normal conditions the excretion was the same in both groups. Under conditions of stress (operation, infection, etc.) the values for both groups rose. However, the rise in hypertrichotic women was much greater, resulting in values which were high even for males (154). Of even greater importance is Koets's finding that in idiopathic hypertrichosis there is a rhythmic increase in the excretion of 17-ketosteroids with every ovulation; in normal women the 17-ketosteroid level remains constant throughout the menstrual cycle (249). This observation has been confirmed and extended by Merivale (293). He pointed out that the cyclic fluctuations in 17-ketosteroid

excretion in benign hypertrichosis were related to the peaks of pregnanediol excretion during the luteal phase of the cycle. No cyclic fluctuation occurred when there was no peak in the pregnanediol excretion. No evidence of an increased suprarenal activity with respect to other steroids could be demonstrated. These findings may indicate the possibility of an abnormal progesterone metabolism, with an exaggerated conversion of progesterone to 17-ketosteroids. The existence of this hypothetical pathway still lacks definite proof. Abnormal progesterone metabolism has also been postulated by Greene (180).

X. EXCRETORY FUNCTION OF HAIR

Hair provides a unique medium for studies of excretion. The long life-span, the coherent hard keratinous structure of hair, and the absence of metabolic turnover in the hair shaft permit most incorporated inorganic materials to be retained for longer periods of time than in most other tissues or secretions of the body.

Because of the great reactivity of heavy metals with sulfhydryl groups of follicular proteins, these elements have a special affinity for hair. They may combine with the sulfhydryl groups of the proteins of the matrix cells, as in the case of selenium, copper, or arsenic. In this way the heavy metals become incorporated in the keratin molecule. From a teleological point of view, this combination may be regarded as a means of detoxification, as the intactness of the sulfhydryl groups of the hair follicles is not essential for the well-being of the organism. When, in cases of severe poisoning, the follicle is confronted with an excess of heavy metals, the formation of organized keratin may break down, and hair loss may result (p. 632).

The mode of chemical attachment of other elements, such as magnesium, calcium, or silicon, is not understood. With these ubiquitous substances, the possibility of extraneous contamination is always present. Because of the difficulty of completely removing dust from the surface of the hair,

The absence of an increased adrenal function in idiopathic hypertrichosis was indicated by the finding that, while cortisone reduced the excretion of 17-ketosteroids and frequently lessened the extent of hirsutism in congenital adrenal hyperplasia (478-80), no such effect was noted in essential hypertrichosis (37).

It is obvious that the problem of essential hypertrichosis requires a great deal of further study. It is often a familial condition, and, therefore, there may be a significant genetic factor. In addition, virilizing factors of different origin may operate in different cases.

there is always doubt as to whether or not in some determinations extraneous material has been included in the reported values. Such external contamination may also account for some of the large individual variations in the results obtained (Table 9).

A. ARSENIC

From the medicolegal point of view, the excretion of arsenic in the hair is of paramount importance in the diagnosis of arsenical poisoning. Keratin has a special affinity for arsenic; it takes up more arsenic, weight for weight, than other tissues do (399). Hair is one of the main channels for the excretion of arsenic; only liver approximates hair in its arsenic content, provided that death occurs within the first few days after poisoning. In poisoned dogs, hair contained six to eight times as much arsenic as did the liver (6).

The main difficulty in establishing whether or not poisoning has taken place lies in the fact that keratin may combine with arsenic from "external" as well as from "internal" sources (110), and there is no chemical method available which would enable us to distinguish between these two types of arsenic (496). The hair may take up arsenic from dust (429) or from other environmental sources (399). When hair is soaked in a solution containing arsenic, within 3 days the concentration of arsenic in the hair becomes

TABLE 9
INORGANIC CONSTITUENTS OF HUMAN HAIR
EXPRESSED IN MG/KG HAIR*

Element	Amounts	Refer ence
Copper.....	28.8-108.0	17
	13-44	261
	31.2-128	174
	21.7-51.5	123
	4.3-6.2 (white hair)	495
	7.0-8.9 (black hair)	495
Iron.....	7.2-12.5	495
	126.0-170.0	17
	0.84-11	174
	9.2-49.5 (av. 27.1) (black hair)	123
	22.1-28.7 (av. 24.3) (blond hair)	123
	17.9-44.0 (gray and white hair)	123
Zinc.....	67.1-132 (av. 97.8) (red hair)	123
	116.0-420.0	17
	64-562	174
	9	367
	255 (84-444) (scalp hair)	126
Manganese..	149 (beard)	126
	197 (pubic hair)	126
	145.1	303
	25-46	17
Aluminum..	12-16	289
	0.00001 or less	174
	26-36	17
Magnesium..	Less than 1	288
	0.00002 or less	174
	10-101	174
Lead.....	21-284.0	17
	20.8-180	19
	30.6	18
	17	254
Nickel.....	52-62 (adults)	431
	29-66 (children)	431
	56 (infants)	431
Cobalt.....	5.4-8.2	17
Silicon.....	14.2-18.1	17
	150.4-188.0	17
Calcium....	300-3,600	174
	700-4,900	174
	188.0-267.2	17
	260-740 (children)	222
Boron.....	320-1,570 (adults)	222
Titanium...	2-8	174
Strontium...	0.032-0.064	174
Silver.....	0.000022-0.091	174
Uranium...	None	17
Bromine....	0.0048-0.045	174
Chromium...	0.000127	215
	0.002-0.007	463
	2	188

* For older data in the literature consult Rothman and Schaaf (370).

seven times that of the solution; nails take up even more of the element (399). The arsenic uptake from such a solution may last for several weeks (496). When, simultaneously with the arsenic of external origin, there is arsenic of internal origin in the hair, it is impossible to remove all the "external" arsenic without extracting considerable amounts of the "internal" arsenic as well. For this reason, methods of cleansing hair after poisoning with arsenic prior to chemical determination are controversial (399, 496). Thus washing for 15 days in water removes only 40 per cent of arsenic absorbed after death (399). According to some authors, the only permissible method consists of soaking the hair in water and alcohol for a few hours (496).

The great chemical affinity between arsenic and hair accounts for its uptake by the hair from the outside and explains the early accumulation in large quantities of this element in cases of systemic poisoning. In guinea pigs, arsenic could be demonstrated in the hair 2 days after poisoning (496). In man, increased amounts of arsenic may be present in the hair at 2 (496), 3 (153), or 5 days from the time of poisoning (6, 110). Young and Smith recovered as much as 770 γ of arsenic per 100 gm. in the proximal portion of the hair as early as 30 hours after excessive intake (497). No arsenic could be demonstrated in the hair 6-8 hours after poisoning (6). Considering the fact that it takes several days before the newly formed hair emerges above the skin surface, the early appearance of arsenic in the proximal part of the hair has been explained by assuming that the arsenic excreted in the sweat or sebum is taken up by the proximal portion of the hair (496).

The real increase in the piliary excretion of arsenic occurs at a later stage. This increase is due to the incorporation of arsenic by the growing hair. Hairs on different parts of the body may excrete arsenic at different rates; in the beard the greatest rise in the excretion occurs 1-2 weeks after poisoning; on scalp hair, after 2-4 weeks (390). Excretion continues for a long time, even if the

intake of arsenic is discontinued, but at a slower rate (153). The excretion may be temporarily increased by administration of sodium thiosulfate (6).

This gradual increase and subsequent tapering-off of the excretion may be well demonstrated by cutting the hairs into segments of equal lengths and determining the arsenic content simultaneously in each portion (462). This method may be used to determine the approximate date of the poisoning, by dividing the length of hair where the maximum amount of arsenic was found by the growth rate of the hair. This method is necessarily crude, because of the different rates of growth in different individuals (462); moreover, the growth rate may very well be influenced by the poisoning (184). Some authors claim that histochemical methods are better suited for pinpointing the date of poisoning than direct chemical methods. In such studies arsenic has been found to occur mostly in the outer cortex and cuticle of the hair (184). Because of the long growth periods of scalp hair, arsenic may be demonstrable many years after poisoning, longer than in any other organ (6, 497).

The amounts of arsenic in the hair vary a great deal, depending on the severity of the poisoning, the time elapsed between the poisoning and the determination, and the length and type of hair used for the determination (6, 496, 429, 497, 390, 391, 494). A modern method for the determination of arsenic consists of making the arsenic radioactive by bombardment with neutrons and determining the amount by comparing the radioactivity with the radioactivity induced in normal hair (182, 183). Because of the ever present possibility of contamination from external sources (496, 471), it is impossible to establish any absolute figure for normal values. A fairly satisfactory figure has been proposed by Young and Smith; these authors consider the arsenic content of hair normal when it is below 100 γ per 100 gm. (497). Anything above this figure should make one suspect the possibility of poisoning.

B. COPPER

The important role of copper in pigment formation and keratinization has been discussed in other chapters (pp. 530 and 371). The high copper content of the wool root (two to ten times higher than in the mature fiber) is related to the essential role of the metal in hair formation (128).

Copper from external sources may combine chemically with the keratin of hair. When hair is immersed in a solution of copper salts, it may take up as much as 12 per cent metal in 10 days. Copper from such external sources combines with cystine. In this process the disulfide bridges are broken, and about half the cystine may be attacked within 10 days. The reaction requires the presence of water and oxygen. X-ray diffraction studies of the contaminated hair do not reveal any crystalline structure in the deposited copper. The hair which has taken up copper in this way assumes a rusty color. Within a few weeks this color turns green, because of the oxidation of the cuprous compounds to cupric compounds within the hair. This oxidation is accelerated by light and humidity. Similar reactions account for the green color of the hair in copper workers (384).

C. IRON

Under experimental conditions iron could be absorbed by the hair of rabbits from solutions of its salts (68). As mentioned elsewhere (p. 518), iron is an essential component of the pigment of human red hair, and red hair contains considerably higher amounts of iron than does hair of any other color (123).

D. ZINC

Zinc is always present in hair in relatively high concentrations. It is not known whether or not the element is essential in the process of hair formation. Certain findings are suggestive of such a role. Thus the zinc content of black dog hair was found to be higher than that of any other organ (371). On a zinc-free diet the growth of hair is impaired in the mouse and rat (p. 623). In zinc poisoning most of the piliary zinc appears in the cuticle, very little in the cortex, and

none in the medulla (392). Zinc is also excreted by the keratin of the epidermis and nails (392, 126).

E. LEAD

Lead is a normal constituent of human hair. It may enter the hair from internal as well as external sources. When lead acetate was fed to guinea pigs for 10 weeks, the lead content of the hair rose by about 3 per cent of the amount of lead ingested. The hair is believed to take up at least as much, if not more, lead than the rest of the body does (254).

Excessive lead intake during pregnancy raises the lead content in the hair of the newborn to about forty times the normal values. The average lead content of the hair of these infants who suffered from cerebral symptoms of lead poisoning ("serous meningitis") was 2.19 gm/kg; the normal value is 56 mg/kg. The lead content in the hair of the mothers rose to a lesser extent (from the normal value of 62 mg/kg to 1.5 gm/kg). Similar selective uptake in the fetal hair has also been observed in the goat (431).

In workers exposed to lead, most of the metal enters the hair from the outside. This external lead apparently combines chemically with the keratin of the hair, because it cannot be removed even by prolonged washing with petroleum ether or soap. When hair is immersed in a solution of lead acetate, its lead content is doubled even in solutions as dilute as 1:1,000. High values of lead in the hair of lead workers are an index of the prevailing hygienic conditions and are not necessarily indicative of poisoning (254).

Indian authors claimed that lead played a role in pigment formation. This belief was based on unusually high lead values in the black hair of Hindu women (17, 18). Eventually these findings have been traced to the use of lead dyes or combs (19, 254). Experimental lead poisoning caused loss of hair in animals (420, 53).

F. SELENIUM

Selenium is deposited in the hair in large amounts after poisoning. Relatively high

values have been reported to occur in the hair of cats (1–3 mg/kg) and of rabbits after poisoning. The increases persisted for 6 weeks after poisoning. On a dry-weight basis the concentration of selenium in the hair was higher than on a wet-weight basis in other organs (473).

In view of the great affinity of selenium for sulfhydryl compounds and cysteine, it is likely that selenium enters into a chemical combination with keratin (307). In chronic selenium poisoning of cattle (alkali disease), the earliest and most common sign is loss of hair from the tails and manes of horses (hence the popular term "bobtailed disease") (307), from the switch of the cow and the body of the hog (334). Coarse and rough hair has been observed in experimental selenium poisoning of dogs (307). The curative effect of bromobenzene in selenium poisoning (259, 260) is also indirect evidence in favor of the assumption that selenium combines with keratin. Bromobenzene, excreted in the hair of cystine-deficient animals (see below), is believed to displace selenium from its combination with cystine.

Whether or not a combination with keratin may occur from externally applied selenium as well has not been investigated. In this respect, the use by Napoleon of selenium-containing baths in the treatment of disorders of keratinization among his troops is of interest (430). A modern form of selenium therapy is the treatment of seborrheic dermatitis and seborrhea sicca with selenium disulfide shampoo (397, 398). In view of the great affinity of selenium for keratinized structures, the advisability of using selenium compounds in the treatment of diseases of the scalp must be questioned. Even when a relatively nonreactive compound, such as selenium disulfide is used, the liberation of selenium ions cannot be excluded with certainty.

G. BROMOBENZENE

When administered to rats on a normal diet, bromobenzene, a sulfhydryl inhibitor, appears in the hair to the same extent as in other tissues. The piliary concentrations,

however, can be greatly increased over those occurring in the liver or muscle by placing the animal on a diet deficient in cystine. It has been suggested that in these deficient animals bromophenylcysteine, the addition product of cysteine and bromobenzene, is incorporated in the hair in an attempt to compensate for the lack of available cystine. These experiments may, at least theoretically, open the way for the development of fungicidal preparations which would act while being excreted with the growing hair (405). The use of bromobenzene for the

elimination of selenium in chronic poisoning has been mentioned above.

H. TRACE ELEMENTS

Nonphysiological heavy metals, such as gold (67), mercury (421), and thallium (16), have been demonstrated in hair after systemic administration. Fluorine is not present under normal conditions but may be excreted in the hair, following its oral administration (406). Antimony and bismuth do not occur in the hair under normal conditions (17).

BIBLIOGRAPHY

- ABELIN, I. Ueber den Nähr- und Wachstumswert des Zwiebacks, *Ztschr. f. Kinderh.*, **50**:465-81, 1930.
- ADAMS, R., and JOHNSON, J. L. Leucenol. VI. A total synthesis, *J. Am. Chem. Soc.*, **71**:705-8, 1949.
- AHMANN, C. F. Hair eating in rabbits, *Florida Agr. Exper. Sta.*, 45th Ann. Rept., p. 85, 1931.
- ALBRIEUX, A. S., and FOURNIER, J. C. M. The local action of testosterone propionate on the development of axillary hair in man, *J. Clin. Endocrinol.*, **9**:1434-35, 1949.
- ALBRIGHT, F.; SMITH, P. H.; and FRASER, R. A syndrome characterized by primary ovarian insufficiency and decreased stature. Report of 11 cases with a digression on hormonal control of axillary and pubic hair, *Am. J. M. Sc.*, **204**:625-48, 1942.
- ALTHAUSEN, T. L., and GUNTHER, L. Acute arsenic poisoning. A report of 2 cases and a study of arsenic excretion with especial reference to hair, *J.A.M.A.*, **92**:2002-6, 1929.
- AMARANTE, A. Sobre um caso de alopecia mixoedematosa curado com o tratamento tiroidiano, *Hospital, Rio de Janeiro*, **19**:441-43, 1941.
- ANDERS, W. Die Entwicklung der Mikrosporie in Berlin, *Oeff. Gesundheitsdienst.*, **13**:69-73, 1951.
- ANTOPOL, W., and UNNA, K. Pathologic aspect of nutritional deficiencies in rats. I. Lesions produced by diets free of vitamin B₆ (pyridoxine) and the response to vitamin B₆, *Arch. Path.*, **33**:241-58, 1942.
- APAJALAHTI, L. Ueber die Wirkung der Thyreoidinbehandlung auf die Haut des Myxödemkinds. Histologische Untersuchungen, *Acta Soc. med. Fenn. duodecim, s.B.*, **15**:1-15, 1931.
- AUBRUN, E. A. Lésions alopéciques et exulcéreuses consécutives aux opérations de la deuxième paire rachidienne cervicale, chez le chat, *Compt. rend. Soc. de biol.*, **111**:78-79, 1932.
- . Prurit et hyperesthésie par section nerveuse. Section des trois premiers nerfs cervicaux, *ibid.*, pp. 404-6.
- . Prurit et hyperesthésie par section nerveuse. Section du trijumeau isolée, ou associée à celle des trois premiers nerfs cervicaux, *ibid.*, pp. 464-66.
- . Action vasculaire et action du sympathique dans le prurit par énévation sensitive partielle, *ibid.*, pp. 481-82.
- . Pruritus and hyperesthesia caused by partial sensory denervation. Experimental alopecia. Contribution to the study of alopecia areata, *Arch. Dermat. & Syph.*, **34**:564-67, 1936.
- BACCAREDDA, A. Sul meccanismo dell'alopecia da tallio. Reperto di tallio nei capelli di ricrescita, *Boll. d. Soc. med.-chir., Pavia*, **49**:655-74, 1935.
- BAGCHI, K. N., and GANGULY, H. D. Mineral constituents of human hair, *Ann. Biochem. & Exper. Med.*, **1**:83-86, 1941.
- BAGCHI, K. N.; GANGULY, H. D.; and SIRDAR, J. N. Lead in human tissues, *Indian J. M. Research*, **26**:935-46, 1939.
- . Lead content of human hair, *ibid.*, **27**:777-92, 1940.
- BAKER, B. L. The relationship of the adrenal, thyroid and pituitary glands to

- the growth of hair, *Ann. New York Acad. Sc.*, **53**:690-707, 1951.
21. BALAZS, A., and HOLMGREN, H. J. Quoted by THYRESSON (440).
 22. BALDWIN, I. G., and GAFFORD, J. A. Arrhenoblastoma: case report, *Endocrinology*, **20**:373-82, 1936.
 23. BARAHAL, H. S. The psychopathology of trichotillomania, *Psychoanalyt. Rev.*, **27**:291-310, 1940.
 24. BARRON, E. S. G. Thiol groups of biological importance, *Adv. in Enzymology*, **11**:201-66, 1951.
 25. BARRON, E. S. G.; MEYER, J.; and MILLER, Z. B. The metabolism of the skin. Effect of vesicant agents, *J. Invest. Dermat.*, **11**:97-118, 1948.
 26. BASLER, A. Über die Funktionen des menschlichen Haarkleides, *München. med. Wchnschr.*, **72**:1019-20, 1925.
 27. BECKER, S. W. Concurrent melanosis and hypertrichosis in distribution of nevus unius lateris, *Arch. Dermat. & Syph.*, **60**:155-60, 1943.
 28. BEEK, C. H. A study on extension and distribution of the human body hair, *Dermatologica*, **101**:317-31, 1951.
 29. BEESON, B. B., and PICKETT, W. J. Experimental alopecia. Contribution to the study of alopecia areata, *Arch. Dermat. & Syph.*, **28**:53-60, 1933.
 30. BEHRMAN, H. T. The scalp in health and disease. St. Louis: C. V. Mosby Co., 1952.
 31. BELINFANTE, A. J. G. Nagelafwijkingen en haaruitval na gouddermatitis, *Nederl. tijdschr. v. geneesk.*, **81**:2613-14, 1937.
 32. BENEDICT, F. G., and FOX, E. L. Der Energieumsatz normaler und haarloser Mäuse bei verschiedener Umgebungstemperatur, *Arch. f. d. ges. Physiol.*, **231**:455-82, 1933.
 33. BENGSTON, B. N. Pituitary therapy of alopecia, *J.A.M.A.*, **97**:1355-58, 1931.
 34. BERGMAN, A. Alopecia areata, artificially produced by intravenous injection of quinine hydrochloride and ethyl carbamate, *Arch. Dermat. & Syph.*, **35**:285-86, 1937.
 35. BERTHOLD. Beobachtungen über das quantitative Verhältniss der Nagel- und Haarbildung beim Menschen, *Arch. f. Anat. u. Physiol.*, pp. 156-60, 1850.
 36. BERTIER, L., and BOCQUILLON, P. Accidents cutanés et muqueux graves, brusques et prolongés consécutifs à un traitement par les sels d'or. Alopecie totale persistante avec larges placards de lichen plan, *Bull. Soc. franç. de dermat. et syph.*, **39**:1335-36, 1932.
 37. BISHOP, P. M. F.; BRAY, B. M.; MOWBRAY, R. R. DE; MERIVALE, W. H. H.; and VAUGHAN-MORGAN, J. Effect of cortisone and excretion of 17-ketosteroids in adrenal virilism and simple hirsutism, *Lancet*, **262**:1287-88, 1952.
 38. BISSELL, G. W. Hirsutism, *Ann. New York Acad. Sc.*, **53**:742-48, 1951.
 39. BISSELL, G. W., and WILLIAMS, R. H. Hirsutism in females: a clinical study of its etiology, course and treatment, *Ann. Int. Med.*, **22**:773-805, 1945.
 40. BISSONNETTE, T. H. Relations of hair cycles in ferrets to changes in anterior hypophysis and to light cycles, *Anat. Rec.*, **63**:159-68, 1935.
 41. BLOCK, W. D., and LEWIS, H. B. The amino acid content of cow and chimpanzee hair, *J. Biol. Chem.*, **125**:561-70, 1938.
 42. BLUMENSAAT, C. Beitrag zur Frage der Alopecia, *Dermat. Wchnschr.*, **120**:106-7, 1949.
 43. BORBERG, A. Et tilfaelde af hirsutisme Cushings syndrome. (Verificeret basofilt Hypofiseadonom) (hirsutism probably due to Cushing's syndrome), *Ugesk. f. laeger*, **102**:709-11, 1940.
 44. BRAUN, H. A.; LUSKY, L. M.; and CALVERY, H. O. Efficacy of 2,3-dimercaptopropanol (BAL) in the therapy of poisoning by compounds of antimony, bismuth, chromium, mercury and nickel, *J. Pharmacol. & Exper. Therap. (suppl.)*, **87**:119-25, 1946.
 45. BROSTER, L. R. Hypertrichosis; a report of 3 cases, *Brit. M. J.*, **1**:1171-74, 1950.
 46. BROWN, H., and KLAUDER, J. V. Sulfur content of hair and of nails in abnormal states. Therapeutic value of hydrolyzed wool. I. Hair, *Arch. Dermat. & Syph.*, **27**:584-604, 1933.
 47. BROWN, W. H., and HOWARD, M. Influence of light environment on the growth of hair in normal rabbits with especial reference to the action of neon light, *J. Exper. Med.*, **48**:57-64, 1928.
 48. BRUNNER, M. J.; RIDDLE, J. M.; and BEST, W. R. Cutaneous side effects of ACTH, cortisone and pregnenolone therapy, *J. Invest. Dermat.*, **16**:205-10, 1951.
 49. BRUSTIER, V.; BOURBON, P.; and VIGNES, R. Sur la localisation de l'arsenic dans les

- cheveux, *Ann. pharmacol. franç.*, **5**:435-37, 1947.
50. BULLIARD, H. Influence de la section et du rasage répété sur l'évolution du poil, *Ann. de dermat. et syph.*, **4**:386-91, 1923.
 51. ———. Recherches sur la croissance des poils chez un homme, *Bull. et mém. Soc. d'anthropol. de Paris*, **7**. VII. 1921. Quoted by F. PINKUS (349).
 52. BUSCHKE, A. Ueber die biologischen Wirkungen und die praktisch-medizinische und soziale Bedeutung des Thalliums, *Dermat. Ztschr.*, **77**:186-222, 1938.
 53. BUSCHKE, A., and BERMAN, L. Ueber chemische und biologische Beziehungen zwischen Thallium und Blei, *Klin. Wchnschr.*, **6**:2428-29, 1927.
 54. BUSCHKE, A., and GUMPert, M. Zur Kenntnis des Sexualcharakters des Kopfhaarkleides, *Klin. Wchnschr.*, **5**:18-20, 1926.
 55. BUSCHKE, A., and PEISER, B. Die Wirkung des Thalliums auf das endokrine System, *Klin. Wchnschr.*, **1**:995, 1922.
 56. ———. Thalliumalopecie und Sinneshaare, *ibid.*, **5**:977-79, 1926.
 57. ———. Die biologischen Wirkungen und die praktische Bedeutung des Thalliums, *Ergebn. d. allg. Path. u. path. Anat.*, **25**:1-57, 1931.
 58. BUSH, I. E. Species differences in adrenocortical secretion, *J. Endocrinol.*, **9**:95-100, 1953.
 59. BUTCHER, E. O. The hair cycles in the albino rat, *Anat. Rec.*, **61**:5-19, 1934.
 60. ———. The effects of irritants and thyroxine on hair growth in albino rats, *Am. J. Physiol.*, **129**:553-59, 1940.
 61. ———. The oxygen consumption of the skin during the hair cycle in the white rat, *ibid.*, **138**:408-11, 1943.
 62. ———. Hair growth and sebaceous glands in skin transplanted under the skin and into the peritoneal cavity in the rat, *Anat. Rec.*, **96**:101-8, 1946.
 63. BUTCHER, E. O., and GROKOEST, A. W. The influence of tissue fluid on hair growth, *Growth*, **5**:175-81, 1941.
 64. CALDINE, D. Le poil, symbole de la force; les ancêtres de nos poilus, *Chron. méd.*, **22**:227-33, 259-69, 1915.
 65. CALLAWAY, J. L.; WORTHAM, J. T.; HAMBLIN, E. C.; and SALMON, A. A. Essential hirsutism. Dermatologic and endocrinologic considerations, *Arch. Dermat. & Syph.*, **60**:528-42, 1949.
 66. CANNON, W. B.; NEWTON, H. F.; BRIGHT, E. M.; MENKIN, V.; and MOORE, R. M. Some aspects of the physiology of animals surviving complete exclusion of sympathetic nerve impulses, *Am. J. Physiol.*, **89**:84-107, 1929.
 67. CASAZZA, R. Ricerche sulla fissazione dell'oro nella cute, mediante l'indagine spettrografica, *Boll. d. Soc. med.-chir.*, Pavia, **48**:415-40, 1934.
 68. CASPE, I. J., and AUSTIN, W. E. Fixation of metal ions by animal hair, *J. Tech. A. Fur Indust.*, **4**:12-18, 1933.
 69. CASTLE, W. E. The furless rabbit, *J. Hered.*, **24**:80-86, 1933.
 70. CERUTTI, P. Ricerche sulla pigmentazione della cute dei conigli, *Gior. ital. di dermat. e sif.*, **71**:1281-86, 1930.
 71. CHAMBERS, G. K. The growth of Angora rabbit hair, *World's Poultry Cong. (sec. F)*, **4**:964-69, 1930.
 72. CHANG, H. On the site of action of thallium, *Chinese J. Physiol.*, **5**:79-84, 1931.
 73. CHASE, H. B. Greying induced by X-rays in the mouse, *Genetics*, **31**:213-14, 1946.
 74. ———. Effects of X-ray doses on the controlled greying response in mice, *Acta Unio internat. contra cancerum*, **6**:768-70, 1949.
 75. ———. Unpublished experiments.
 76. ———. Greying of hair. I. Effects produced by single doses of X-rays on mice, *J. Morphol.*, **84**:57-80, 1949.
 77. CHASE, H. B., and MONTAGNA, W. Relation of hair proliferation to damage induced in the mouse skin, *Proc. Soc. Exper. Biol. & Med.*, **76**:35-37, 1951.
 78. CHASE, H. B.; RAUCH, H.; and SMITH, V. W. Critical stages of hair development and pigmentation in the mouse, *Physiol. Zoöl.*, **24**:1-8, 1951.
 79. CHAVARRIA, A. P.; GOLDMAN, L.; SAENZ-HERRERA, C.; and CARVAJAL-CORDERO, E. Canities and alopecia in children associated with avitaminosis, *J.A.M.A.*, **132**:570-72, 1946.
 80. CHIEFFI, M. Effect of testosterone administration on the beard growth of elderly males, *J. Gerontol.*, **4**:200-204, 1949.
 81. CHRIST, J. Die Pluri- und die Terminalinnervation als biologisch wirksame Faktoren, im besonderen als anatomische Grundlagen der Prädilektionsstellen des

- Ulcus rodens bzw. der beginnenden Scheitelglatze; über Beziehungen der Innervation, vor allem der Pluriinnervation, zu Hauteigentümlichkeiten bei Mensch und Tier, sowie über die Morphogenese des Scheitelwirbels, Ztschr. f. Konstitutionslehre, **18**:262-86, 1934.
82. CLARK, F. H.: Postjuvenile nude in the deermouse, J. Hered., **30**:213-15, 1939.
 83. CLAY, R. C.; COOK, K.; and ROUTH, J. I. Studies in the composition of human hair, J. Am. Chem. Soc., **62**:2709-10, 1940.
 84. COLLINS, H. H. Studies of normal moult and artificially induced regeneration of pelage in *Peromyscus*, J. Exper. Zoöl., **27**:73-99, 1918.
 85. ———. Studies of the pelage phases and of the nature of color variations in mice of the genus *Peromyscus*, *ibid.*, **38**:45-94, 1923.
 86. COOPER, Z. K. Relation of endocrine glands to growth and distribution of hair. Review of literature, Arch. Dermat. & Syph., **21**:1007-29, 1930.
 87. ———. The effect of thallium acetate on thyroidectomized albino rats, *ibid.*, **25**:522-27, 1932.
 88. COOPER, Z. K., and ENGMAN, M. F. A study of the stimulating effect of small doses of thallium acetate on the rate of the growth of hair in the albino rat, Arch. Dermat. & Syph., **23**:1031-40, 1931.
 89. COSACESCO, A.; DRAGANESCO, S.; GEORGESCO, M.; and DINISCHIOTU, G. T. Lutéinome de l'ovaire: contribution anatomo-clinique à l'étude du virilisme ovarien, Presse méd., **39**:1264-67, 1931.
 90. CRAFT, W. A., and BLIZZARD, W. L. The inheritance of semi-hairlessness in cattle, J. Hered., **25**:385-90, 1934.
 91. CREW, F. A. E., and MIRSKAIA, L. The character "hairless" in the mouse, J. Genetics, **25**:17-24, 1931.
 92. CSILLAG, J. Ueber Berufshypertrichose, Arch. f. Dermat. u. Syph., **134**:147-50, 1921.
 93. ———. Beschäftigungshypertrichose, Dermat. Wchnschr., **72**:603, 1921.
 94. CUNHA, T. J.; KIRKWOOD, S.; PHILLIPS, P. H.; and BOHSTEDT, G. Effect of inositol on rat alopecia, Proc. Soc. Exper. Biol. & Med., **54**:236-38, 1943.
 95. CUNHA, T. J.; LINDLEY, D. C.; and ENSMINGER, M. E. Biotin deficiency in pigs fed desiccated egg white, J. Animal Sc., **5**:219-25, 1946.
 96. CUNNINGHAM, I. J. Influence of dietary iron in hair and wool growth, New Zealand J. Agr., **44**:335-37, 1932.
 97. DAMM, G. Zur Behandlung der Alopecia areata durch Hypophysentransplantation, Med. Klin., **44**:1153-55, 1949.
 98. DANFORTH, C. H. Studies on hair; with special reference to hypertrichosis, Arch. Dermat. & Syph., **11**:494-508, 637-53, 804-21, 1925.
 99. ———. The hair, Natural Hist., **26**:75-79, 1926.
 100. ———. A case of alopecia in the fowl, J. Hered., **19**:547-50, 1928.
 101. ———. Physiology of human hair, Physiol. Rev., **19**:94-111, 1939.
 102. DAVID, L. T. Hairless mammals. Comparative histologic studies; preliminary report, Arch. Dermat. & Syph., **24**:196-203, 1931.
 103. ———. Histology of the skin of the Mexican hairless swine (*Sus scrofa*), Am. J. Anat., **50**:283-92, 1932.
 104. ———. Studies on the expression of genetic hairlessness in the house mouse (*Mus musculus*), J. Exper. Zoöl., **68**:501-18, 1934.
 105. DAWSON, H. L. A study of hair growth in the guinea pig (*Cavia cobaya*), Am. J. Anat., **45**:461-84, 1930.
 106. ———. Hair growth and pregnancy, Science, **71**:607, 1930.
 107. ———. On hair growth. A study of the effect of pregnancy on the activity of the follicle in the guinea pig (*Cavia cobaya*), Am. J. Anat., **53**:89-115, 1933.
 108. DAY, H. G., and SKIDMORE, B. E. Some effect of dietary zinc deficiency in the mouse, J. Nutrition, **33**:127-38, 1947.
 109. DEBIDOUR-MONRAD, H., and LASSNER, J. Observations cliniques sur le traitement des plaies par l'acide oxalique et découverte de son action sur le développement du système pileux, Union méd. du Canada, **74**:589-93, 1945.
 110. DÉROBERT, L., and LE BRETON, R. Valeur de la présence d'arsenic dans les cheveux pour l'estimation de l'intoxication arsenicale, Ann. de méd. lég., **31**:172-76, 1951.
 111. DIEKE, S. H. Pigmentation and hair growth in black rats, as modified by the chronic administration of thiourea, phenyl-

thiourea and alpha-naphthylthiourea, *Endocrinology*, **40**:123-36, 1947.

112. ———. Effects of removing various endocrine glands on the hair cycles of black rats, *ibid.*, **42**:315-19, 1948.
113. DILLAHA, C. J., and ROTHMAN, S. Treatment of alopecia areata totalis and universalis with cortisone acetate, *J. Invest. Dermat.*, **18**:5-6, 1952.
114. ———. Therapeutic experiments in alopecia areata with orally administered cortisone, *J.A.M.A.*, **150**:546-50, 1952.
115. DOUGLASS, M. Masculinizing tumor of the ovary of the adrenal type, *Am. J. Obst. & Gynec.*, **53**:190-98, 1947.
116. DOUPE, J., and SHARP, M. E. Studies in denervation. G. Sebaceous glands, *J. Neurol. & Psychiat.*, **6**:133-35, 1943.
117. DREYFUS, G.; ZARA, M.; and ALEXANDRE, C. Hirsutisme féminin et virilisme pileaire, *Semaine d. hôp. Paris*, **27**:1398-1404, 1951.
118. DRY, F. W. The coat of the mouse (*Mus musculus*), *J. Genetics*, **16**:287-340, 1926.
119. DUGGINS, O. H., and TROTTER, M. Age changes of head hair from birth to maturity. II. Medullation in hair of children, *Am. J. Phys. Anthropol.*, **8**:399-415, 1950.
120. ———. Changes in morphology of hair during childhood, *Ann. New York Acad. Sc.*, **53**:569-75, 1951.
121. DUPERTUIS, C. W.; ATKINSON, W. B.; and ELFTMAN, H. Sex differences in pubic hair distribution, *Human Biol.*, **17**:137-42, 1945.
122. DURWARD, A., and RUDALL, K. M. Studies of hair growth in the rat, *J. Anat.*, **83**:325-35, 1949.
123. DUTCHER, T. F., and ROTHMAN, S. Iron, copper and ash content of human hair of different colors, *J. Invest. Dermat.*, **17**:65-68, 1951.
124. EATON, P., and EATON, M. W. Temperature and hair growth, *Science*, **86**:354, 1937.
125. EBSTEIN, E. Zur Etymologie der Alopezie, *Dermat. Wchnschr.*, **81**:1461-62, 1925.
126. EGGLESTON, W. G. E. The zinc content of epidermal structures, *Chinese J. Physiol.*, **13**:399-404, 1938.
127. EICHHOLZ, E. Experimentelle Versuche über die Anregung des Haarwuchses durch äussere Behandlung, *Dermat. Wchnschr.*, **88**:161-70, 1929.
128. ELLIS, W. J.; GILLESPIE, J. M.; and LINDLEY, H. Biochemical studies of the wool root, *Nature*, **165**:545-48, 1950.
129. ELVEHJEM, C. A., and HART, E. B. Lack of zinc prevents normal hair growth in rats, *Wisconsin Agr. Exper. Sta. Bull.*, **428**:23-24, 1934.
130. EMERSON, G. A., and KERESZTESY, J. C. Biotin deficiency in the rat, *Proc. Soc. Exper. Biol. & Med.*, **51**:358-61, 1942.
131. ENGELBACH, W. Studies on hair growth and pigmentation, *Texas State J. Med.*, **23**:7-20, 1927.
132. EPSTEIN, A. L. Somatologische Studien zur Psychiatrie, *Ztschr. f. d. ges. Neurol. u. Psychiat.*, **140**:124-51, 1932.
133. ERSHOFF, B. H., and DEUEL, H. J., JR. Inadequacy of lactose and β -lactose as dietary carbohydrates for the rat, *J. Nutrition*, **28**:225-34, 1944.
134. EULER, B. VON; EULER, H. VON; and SÄBERG, I. Symptoms of dermatitis and loss of hair during lactoflavin deficiency and fat-free diet, *Naturwiss.*, **30**:266, 1942.
135. FARBER, S. Lecture given at the February 10 and 11, 1950, meeting of the New York Academy of Sciences.
136. FASAL, H. Zur Beeinflussung des Haarwachstums, *Wien. med. Wchnschr.*, **82**:1080-83, 1932.
137. ———. Haarausfall als Symptom der Vermännlichung, *Dermat. Ztschr.*, **74**:249-54, 1936.
138. FINKLER, R. S. Estrogenic therapy in hypertrichosis with secondary amenorrhea, *M. Woman's J.*, **50**:281-84, 1943.
139. FIOCCO, G. B. Tallio ed alopecia areata, *Gior. ital. di dermat. e sif.*, **66**:1287-88, 1925.
140. FLESCH, P. Inhibitory action of extracts of mammalian skin on pigment formation, *Proc. Soc. Exper. Biol. & Med.*, **70**:136-40, 1949.
141. ———. Hair loss from squalene, *ibid.*, **76**:801-3, 1951.
142. ———. Experimental loss of hair, *J. Soc. Cosmet. Chem.*, **3**:84-89, 1952.
143. ———. Inhibition of keratin formation with unsaturated compounds, *J. Invest. Dermat.*, **19**:353-63, 1952.
144. ———. Hair loss from sebum, *Arch. Dermat. & Syph.*, **67**:1-9, 1953.
145. ———. Studies on the mode of action of vitamin A, *J. Invest. Dermat.*, **21**:421-34, 1953.
146. FLESCH, P., and GOLDSTONE, S. B. Effect

- of thallium on sulfhydryl compounds in vitro, *J. Invest. Dermat.*, **15**:345-47, 1950.
147. FLESCH, P., and GOLDSTONE, S. B. Depilatory action of the intermediary polymers of chloroprene, *Science*, **113**:126-27, 1951.
 148. ———. Local depilatory action of unsaturated compounds. The effect of human sebum on hair growth, *J. Invest. Dermat.*, **18**:267-87, 1952.
 149. FLESCH, P., and HUNT, M. Local depilatory action of some unsaturated compounds, *Arch. Dermat. & Syph.*, **65**:261-69, 1952.
 150. FLYNN, R. Hirsutism of adrenal origin, *M. J. Australia*, **1**:11-19, 1941.
 151. ———. Hirsutism of adrenal origin, *ibid.*, **2**:519-22, 1942.
 152. FOLLIS, R. H.; DAY, H. G.; and MCCOLLUM, E. V. Histological studies of the tissues of rats fed a diet extremely low in zinc, *J. Nutrition*, **22**:223-37, 1941.
 153. FONZÈS-DIACON. Élimination de l'arsenic par les cheveux, *Ann. de méd. lég.*, **15**:793-94, 1935.
 154. FORBES, A. P.; DONALDSON, E. C.; REIFENSTEIN, E. C.; and ALBRIGHT, F. The effect of trauma and disease on the urinary 17-ketosteroid excretion in man, *J. Clin. Endocrinol.*, **7**:264-88, 1947.
 155. FORSSBERG, A. On the possibility of protecting the living organism against roentgen rays by chemical means, *Acta radiol.*, **33**:296-304, 1950.
 156. FORSTER, A. Beeinflussung des Haarwachstums durch äusserliche Mittel, *Arch. f. exper. Path. u. Pharmacol.*, **144**:363-71, 1929.
 157. FRAENKEL, L. Thecoma and hyperthecosis of the ovary, *J. Clin. Endocrinol.*, **3**:557-59, 1943.
 158. FREUD, J. Hypophysis and hair, *Acta brev. Neerland.*, **4**:99-100, 1934.
 159. ———. Continued observations concerning the growth of hair, *ibid.*, **5**:26-27, 1935.
 160. FRIEDENTHAL, H. Das Dauerhaarkleid des Menschen. Jena: G. Fischer, 1908.
 161. FUCHS, H. Ueber die Wachstumsgeschwindigkeit der Haare, *Ztschr. f. Biol.*, **98**:215-20, 1937.
 162. FUHS, H. Studien über die Wachstumsgeschwindigkeit der Kopfhaare unter normalen Bedingungen und bei Anwendung hyperämischer Mittel, *Med. Klin.*, **16**:1320-23, 1920.
 163. GALEWSKY, E. Erkrankungen der Haare und des Haarbodens. In: JADASSOHN, *Handb. d. Haut- u. Geschlechtskr.*, **13/1**:129-436. Berlin: J. Springer, 1932.
 164. GARN, S. M. Types and distribution of the hair in man, *Ann. New York Acad. Sc.*, **53**:498-507, 1951.
 165. GEARY, J. R., JR. Effect of roentgen rays during various phases of the hair cycle of the albino rat, *Am. J. Anat.*, **91**:51-105, 1952.
 166. GERSHBERG, H. Skin transplantation between hairless and haired rats, *Proc. Soc. Exper. Biol. & Med.*, **40**:659-65, 1939.
 167. GIACHETTI, A. Ulteriori contributi alla conoscenza dell'alopecia da dibromotirosina nel ratto albino, *Boll. Soc. ital. biol. sper.*, **25**:183-84, 1949.
 168. ———. Azione della tiroxina e della dibromotirosina sul trofismo pilifero, *ibid.*, pp. 185-86.
 169. ———. L'effetto alopecizzante della 3-5 dibromotirosina, *Arch. di fisiol.*, **48**:230-59, 1949.
 170. GIBBS, H. F. A study of the post-natal development of the skin and hair of the mouse, *Anat. Rec.*, **80**:61-79, 1941.
 171. GLANZMANN, E., and MEIER, K. Pantothenensäure und Schilddrüse, *Ztschr. f. Vitaminforsch.*, **16**:322-38, 1945.
 172. GLASS, S. J., and BERGMAN, H. C. Subclinical adrenogenital syndrome, *Endocrinology*, **23**:625-29, 1938.
 173. GOHLKE and HOLTSCHMIDT. Neurohistologische Studien bei Alopecia areata, *Arch. f. Dermat. u. Syph.*, **191**:527-30, 1950.
 174. GOLDBLUM, R. W.; DERBY, S.; and LERNER, A. B. The metal content of skin, nails and hair, *J. Invest. Dermat.*, **20**:13-18, 1953.
 175. GOLDMAN, L. Acquired achromotrichia and hypotrichosis in children in the southern Ohio area, *Arch. Dermat. & Syph.*, **63**:443-49, 1951.
 176. GOLDMAN, L., and MASON, L. M. Investigational studies in some congenital and acquired defects of hair in children, *J. Invest. Dermat.*, **11**:323-36, 1948.
 177. GOODMAN, H. Thallium acetate. Toxicity and depilatory action, *New York J. Med.*, **32**:1307-13, 1932.
 178. GRANT, R.; CORNBLEET, T.; and GROSSMAN, M. I. Influence of local application of adrenal cortical extract on hair growth in

- the human, *Arch. Dermat. & Syph.*, **62**:717-18, 1950.
179. GREENBLATT, R. B. A digression on sexual hair, *J. M. A. Georgia*, **36**:13-19, 1947.
 180. GREENE, R. Androgen and pregnanediol excretion in hypertrichosis, *Lancet*, **239**:486-87, 1940.
 181. GRICOUROFF, G. Relation entre la mue et la radiosensibilité du pelage chez le lapin, *Compt. rend. Soc. de biol.*, **128**:496-99, 1938.
 182. GRIFFON, H., and BARBAUD, J. Méthode utilisable en toxicologie pour la détection de l'arsenic dans les cheveux, par l'étude de la radioactivité de cet élément provoquée in situ, *Ann. pharm. franç.*, **9**:545-51, 1951.
 183. ———. Utilisation en toxicologie de la radioactivité provoquée de l'arsenic pour la détection et l'étude de la répartition de cet élément dans les cheveux, *Compt. rend. Acad. d. sc.*, **232**:1455-57, 1951.
 184. GRIFFON, H.; DÉROBERT, L.; and CLAVELIN, P. Intoxication aiguë criminelle par l'arsenic. Étude comparative de l'examen histo-chimique et chimique des cheveux, *Ann. de méd. lég.*, **27**:165-68, 1947.
 185. GRIGORY-BRATU, I. Phénomènes du nerf vago-sympathique (chien), *Bull. Fac. Ști. Cernăuți*, **7**:123-28, 1933-34.
 186. GROSS, P.; HARVALIK, Z.; and RUNNE, E. Vitamin deficiency syndrome in the albino rat precipitated by chronic zinc chloride poisoning, *J. Invest. Dermat.*, **4**:385-98, 1941.
 187. GROSS, P.; RUNNE, E.; and WILSON, J. F. Studies on the effect of thallium poisoning of the rat. The influence of cystine and methionine on alopecia and survival periods, *J. Invest. Dermat.*, **10**:119-34, 1948.
 188. GRUSHKO, Y. M. Chromium as a bioelement, *Biokhimiya*, **13**:124-26, 1948.
 189. GSELL, J. L. Therapeutische Wirkung der Pantothensäure (Bepanthen Roche) bei menschlicher Alopecia, *Schweiz. med. Wchnschr.*, **74**:1171, 1944.
 190. GUBNER, R. Effect of aminopterin on epithelial tissues, *Arch. Dermat. & Syph.*, **64**:688-99, 1951.
 191. GUSZMAN, J. Unpublished experiments. Personal communication.
 192. GYÖRGY, P. The biological action of vitamins, pp. 54-72. Chicago: University of Chicago Press, 1942.
 193. HADDOW, A.; ELSON, L. A.; ROE, E. M. F.; RUDALL, K. M.; and TIMMIS, G. M. Artificial production of coat color in the albino rat; its relation to pattern in the growth of hair, *Nature*, **155**:379-81, 1945.
 194. HALE, C. W. Colour and growth of hair in rabbits, *Nature*, **155**:670-71, 1945.
 195. HAMBLIN, E. C.; CUYLER, W. K.; and BAPTIST, M. Urinary excretion of 17-ketosteroids in ovarian failure. I. In hirsutism and virilizing syndromes, *J. Clin. Endocrinol.*, **1**:763-71, 1941.
 196. HAMILTON, J. B. Male hormone stimulation is prerequisite and an incitant in common baldness, *Am. J. Anat.*, **71**:451-80, 1942.
 197. ———. A secondary sexual character that develops in an organ common to both sexes, but normally only in man, with a discussion of the relation of this character to endocrine stimulation, *J. Clin. Endocrinol.*, **7**:465, 1947.
 198. ———. Quantitative measurement of a secondary sex character, axillary hair, *Ann. New York Acad. Sc.*, **53**:585-99, 1951.
 199. ———. Patterned loss of hair in man, type and incidence, *ibid.*, pp. 708-28.
 200. HARDY, M. H. The development of mouse hair in vitro with some observations on pigmentation, *J. Anat.*, **83**:364-84, 1949.
 201. ———. The development of pelage hairs and vibrissae from skin in tissue cultures, *Ann. New York Acad. Sc.*, **53**:546-61, 1951.
 202. HARRIS, H. The relation of hair growth on the body to baldness, *Brit. J. Dermat.*, **59**:300-309, 1947.
 203. HART, E. B., and STEENBOCK, H. Iodine in relation to hairless pig malady, *J. Biol. Chem.*, **33**:313-23, 1918.
 204. HARTWELL, G. A. A possible correlation between dietary protein and loss of fur in young growing rats, *Biochem. J.*, **19**:75-79, 1925.
 205. HAYWARD, W. G., and McCULLA, F. J. Cortical tumors of the adrenal with report of a case, *Urol. & Cutan. Rev.*, **43**:265-69, 1939.
 206. HAZEN, H. H. Post influenzal alopecia, *J.A.M.A.*, **72**:1452, 1919.
 207. HEARD, E. V., and LEWIS, H. B. The metabolism of sulfur. XXV. Dietary methionine as a factor related to the growth and composition of the hair of the

- young white rat, *J. Biol. Chem.*, **123**:203-10, 1938.
208. HELLINGA, G. Hair growth in rats, *Acta brev. Neerland.*, **14**:83-86, 1946.
 209. HEMPELMANN, L. H.; LISCO, H.; and HOFFMAN, J. G. The acute radiation syndrome. A study of nine cases and a review of the problem, *Ann. Int. Med.*, **36**:279-510, 1952.
 210. HERRINGTON, L. P. The role of the piliary system in mammals and its relation to the thermal environment, *Ann. New York Acad. Sc.*, **53**:600-607, 1951.
 211. HEYROTH, F. F. Thallium. A review and summary of medical literature, *Pub. Health Rep. Suppl. No. 197*, 1947.
 212. HINSEY, J. C., and CUTTING, C. C. Observations on nervous factors in hair growth, *Anat. Rec. (suppl.)*, **52**:57, 1932.
 213. HOFF, F. Haarkleid und vegetatives System, *Deutsche med. Wchnschr.*, **75**:478-82, 1950.
 214. ———. Beobachtungen an Hauttransplantaten, *Klin. Wchnschr.*, **31**:56-57, 1953.
 215. HOFFMANN, J. Uran in Anhangsgebilden der Haut bei Mensch und Tier, *Ztschr. f. physiol. Chem.*, **270**:120, 1943.
 216. HOGAN, A. G., and RICHARDSON, L. R. Differentiation of the antidermatitis factor, *Science*, **83**:17-18, 1936.
 217. HOLLANDER, L.; EVANS, G. F.; and KRUGH, F. J. Multiple cutaneous effects of potassium sulfocyanate, *Arch. Dermat. & Syph.*, **59**:112-14, 1949.
 218. HOLMER, A. J. M. Hirsutismus bij cyclisch vloeiende vrouwen, *Nederl. tijdschr. v. geneesk.*, **94**:958-65, 1950.
 219. ———. Hirsutism and hypertrichosis in women with cyclical bleeding, *Acta physiol. et pharmacol. néerl.*, **2**:145-47, 1951.
 220. HOPKINS, J. G.; WELD, J. T.; and HUBER, W. H. Cyst formation, acneform lesions and hair growth following intradermal injection of staphylococci in sensitized rabbits, *J. Invest. Dermat.*, **16**:339-54, 1951.
 221. HURXTHAL, L. M. Sublingual use of testosterone in seven cases of hypogonadism: report of three congenital eunuchoids occurring in one family, *J. Clin. Endocrinol.*, **3**:551-56, 1943.
 222. IKEUCHI, K. Beiträge zur Biochemie der Haare. I. Ueber den Kalziumgehalt der Haare, *J. Orient. Med.*, **7**:1, 1927.
 223. ITALLIE, L. V. The arsenic content of hair, *Pharm. Weekblad.*, **69**:1134-45, 1932.
 224. JACOB, A. Dépilation axillaire au cours des cirrhoses hépatiques, *Presse méd.*, **42**:2076, 1934.
 225. JACOBS, E. C. Effects of starvation on sex hormones in the male, *J. Clin. Endocrinol.*, **8**:227-32, 1948.
 226. JAFFÉ, R. Cholesterinstoffwechsel und Haarwuchs, *Klin. Wchnschr.*, **5**:507-8, 1926.
 227. JALOWY, B. O. Regeneration and development of the nerve endings in the sinus hairs (guinea pigs), *Folia morphol.*, **5**:251-90, 1934-35.
 228. JAUSION, H., and BRIGON, A. Kératogénèse et antistéatose. Essai de traitement des alopecies par les amino-acides soufrés, *Rev. d. sc. méd.*, **4**:85-88, 1951.
 229. JOHN, F. Zur vegetativen Nervenversorgung der menschlichen Haare und Haarmuskeln, *Arch. f. Dermat. u. Syph.*, **183**:1-14, 1942.
 230. JOHNSON, P. L., and BEVELANDER, G. Glycogen and phosphatase in the developing hair, *Anat. Rec.*, **95**:193-97, 1946.
 231. JOHNSON, P. L.; BUTCHER, E. O.; and BEVELANDER, G. The distribution of alkaline phosphatase in the cyclic growth of the rat hair follicle, *Anat. Rec.*, **93**:355-61, 1945.
 232. JONKHOFF, A. R. Über die Bedeutung der Atrophie und Regeneration für den experimentellen Teerkrebs, *Ztschr. f. Krebsforsch.*, **26**:25-31, 1927.
 233. JOSEFSON, A.; FAGERSTRÖM, E.; and BERGSTRAND, H. Two cases of hirsutism (virilism) with ovarian tumors, *Acta med. Scandinav.*, **77**:485-501, 1932.
 234. JOSEPH, M. Experimentelle Untersuchungen über die Aetiologie der Alopecia areata, *Monatsh. f. prakt. Dermat.*, **5**:483-89, 1886.
 235. JOSEPH, W. Der Einfluss des Cholesterinpräparates Trilysin auf den Haarausfall, *Med. Klin.*, **24**:343, 1928.
 236. JUON, M. Résultats cliniques obtenus avec l'acide pantothénique dans les maladies du cuir chevelu, *Dermatologica*, **91**:310-18, 1945.
 237. JUSTER, M. E. Action des fortes doses d'oestrogènes et de testostérone sur la pilosité de deux malades prostatiques, *Bull. Soc. franç. de dermat. et syph.*, **57**:129-30, 1950.

238. KADANOFF, D. Über die Nerven in der äusseren Wurzelscheide der Haare des Menschen, *Ztschr. f. Zellforsch. u. mikr. Anat.*, **6**:631-36, 1928.
239. KARPELIS, E. Zur Behandlung des Haar-ausfalles, *Wien. med. Wchnschr.*, **80**:316, 1930.
240. KARRENBERG, C. L. Nebenerscheinungen nach therapeutischer Verabfolgung von Thallium. Ein Übersichtsreferat, *Zentralbl. f. Haut- u. Geschlechtskr.*, **42**:1-31, 1932.
241. KENNEDY, F. A. Alopecia of beard following phrenicectomy, *J.A.M.A.*, **100**:257, 1933.
242. KEPLER, E. J.; PETERS, G. A.; and MASON, H. L. Addison's disease associated with pubic and axillary alopecia and normal menses, *J. Clin. Endocrinol.*, **3**:497-99, 1943.
243. KLEIPOOL, R. J. C., and WIBAUT, J. P. Mimosine (leucaenine). 5th communication, *Rec. trav. chim.*, **69**:37-44, 1950.
244. KLIGMAN, A. M. Unpublished experiments.
245. KNOWLES, F. C. Hypertrichiasis in childhood: the so-called dog-faced boy, *Pennsylvania M. J.*, **24**:401-4, 1921.
246. KOCH, E. Experimentelle Untersuchungen über entzündung- und nekroseerzeugende Wirkung von *Viscum album*, *Ztschr. f. d. ges. exper. Med.*, **103**:740-49, 1938.
247. KÖNIGSBAUER, H. Experimenteller Beitrag zum Mechanismus der Thalliumepilation bei der weissen Maus, *Arch. f. Dermat. u. Syph.*, **190**:527-30, 1950.
248. KÖNIGSTEIN, H. Über dunkle Flecke auf der Kaninchenhaut und ihre Beziehungen zum Haarwechsel, *Arch. f. Dermat. u. Syph.*, **143**:314-28, 1923.
249. KOETS, P. The excretion of 17-ketosteroids in idiopathic hirsutism, *J. Clin. Endocrinol.*, **9**:795-800, 1949.
250. KOGOJ, F. Hypertrichosis an abgeheilten Psoriasisherden, *Dermat. Wchnschr.*, **104**:402-3, 1937.
251. KOSTERMANS, D. Notes on mimosine, *Rec. trav. chim.*, **65**:319, 1946.
252. ———. The structure of mimosine, *ibid.*, **66**:93-98, 1947.
253. KOZAM, G. Capillary fragility in the skin of the albino rat and its relation to the hair cycle, *Am. J. Physiol.*, **169**:529-36, 1952.
254. KRAUT, H., and WEBER, M. Ueber den Bleigehalt der Haare, *Biochem. Ztschr.*, **317**:133-48, 1944.
255. KYLIN, E. Ueber die hormonale Regulation des Haarwuchses, *Acta med. Scandinav.*, **103**:144-51, 1940.
256. LEBEUF, M. F. Traitement de l'alopecie masculine par l'association hexoestrol-panthénol, *Bull. Soc. franç. de dermat. et syph.*, **56**:76-78, 1949.
257. LE DOUBLE, A. F., and HOUSSAY, F. Les velus dans la science et dans l'histoire, *Aesculape*, **2**:158-66, 1912.
258. LEFÉBURE, M. Innervation des poils chez l'homme, *Bibliog. anat.*, **18**:142-61 and 241-43, 1909.
259. LEMLEY, R. E. Selenium poisoning in the human, *Journal-Lancet*, **60**:528-31, 1940.
260. LEMLEY, R. E., and MERRYMAN, M. P. Selenium poisoning in the human, *Journal-Lancet*, **61**:435-38, 1941.
261. LERNER, A. B. Quoted by GOLDBLUM *et al.* (174).
262. LESPINNE, V. La physiologie de la sécrétion du poil, *Bull. Acad. roy. de méd. de Belgique*, **9**:681-700, 1929.
263. LETARD, E. Chiens nus et hommes chiens, *Rev. de path. comparée*, **34**:1727-41, 1934.
264. ———. Hairless Siamese cats, *J. Hered.*, **29**:173-75, 1938.
265. LEVENTHAL, M. L., and COHEN, M. R. Bilateral polycystic ovaries, the Stein syndrome, *Am. J. Obst. & Gynec.*, **61**:1034-46, 1951.
266. LEVIN, O. L. Post-influenza alopecia, *M. Press*, **107**:47, 1919.
267. ———. Post-influenza alopecia, *New York M. J.*, **109**:409-10, 1919.
268. LIEBOW, A. A. Effects produced on hair by atomic bombs, *Ann. New York Acad. Sc.*, **53**:688-89, 1951.
269. LIEBOW, A. A.; WARREN, S.; and DE COURSEY, E. Pathology of atomic bomb casualties, *Am. J. Path.*, **25**:853-1027, 1949.
270. LIGHT, A. E. Histological study of human scalps exhibiting various degrees of non-specific baldness, *J. Invest. Dermat.*, **13**:53-59, 1949.
271. LIGHTBODY, H. D., and LEWIS, H. B. The metabolism of sulfur. XV. The relation of the protein and cystine content of the diet to the growth of the hair in the white rat, *J. Biol. Chem.*, **82**:485-97, 1929.
272. ———. The metabolism of sulfur. XVI. Dietary factors in relation to the chemical composition of the hair of the young white rat, *ibid.*, pp. 663-71.

273. LINSEY, K. Experimentelle Untersuchungen über das Haarwachstum beim Kaninchen, *Klin. Wchnschr.*, **5**:1490, 1926.
274. LINSEY, K., and KÄHLER, H. Cholesterinstoffwechsel und Haarwuchs, *Klin. Wchnschr.*, **7**:116-18, 1928.
275. ———. Experimentelles zur Thalliumalopecie, *Arch. f. Dermat. u. Syph.*, **155**:175-80, 1928.
276. LÖHNER, L. Ueber Entstehungsgeschichte und Funktionen des menschlichen Haarkleides, *Biol. Zentralbl.*, **44**:384-98, 1924.
277. LOWENSTEIN, P. S. The etiology of hypertrichosis. A review of the literature, *Nation's Health*, **3**:157-61, 1921.
278. LUTZ, W. Zur Kenntnis der biologischen Wirkung der Strahlen auf die Haut, mit spezieller Berücksichtigung der Pigmentbildung, *Arch. f. Dermat. u. Syph.*, **124**:233-96, 1917.
279. LYELL, A., and WHITTLE, C. H. Hypertrichosis lanuginosa, acquired type, *Proc. Roy. Soc. Med.*, **44**:576-77, 1951.
280. MARCHIONINI, A., and DRAESEKE, L. Physikalisch-chemisch nachweisbare Veränderungen der Haare bei Haarkrankheiten und inneren Leiden, *Dermat. Wchnschr.*, **107**:917-22, 1938.
281. MARCHIONINI, A., and WEISS, L. Weitere physikalisch-chemische Untersuchungen an menschlichen Haaren unter physiologischen Bedingungen, *Dermat. Wchnschr.*, **106**:661-66, 1938.
282. MARTIN, G. J. The mouse antialopecia factor, *Science*, **93**:422-23, 1941.
283. MARTIN, G. J., and ANSBACHER, S. Role of paraaminobenzoic acid in vitamin B complex studies with mice, *Proc. Soc. Exper. Biol. & Med.*, **48**:118-20, 1941.
284. MAXWELL, A. F. Masculinizing tumors of the ovary, *West. J. Surg.*, **45**:134-47, 1937.
285. MAY, R. Erfahrungen mit Heparin und Thrombocid in der Thrombosebehandlung, *München. med. Wchnschr.*, **92**:1541, 1950.
286. ———. Thrombocid und Haarausfall, *ibid.*, **93**:1419, 1951.
287. MCCALL, K. B.; WAISMAN, H. A.; ELVEHJEM, C. A.; and JONES, E. S. A study of pyridoxine and pantothenic acid deficiencies in the monkey (*Macaca mulatta*), *J. Nutrition*, **31**:685-97, 1946.
288. MCCOLLUM, E. V.; RASK, O. S.; and BECKER, J. E. A study of the possible role of aluminum compounds in animal and plant physiology, *J. Biol. Chem.*, **77**:753-68, 1928.
289. MCCRAE, J. Manganese in hair, *J. South African Chem. Inst.*, **6**:18-19, 1923.
290. MCGLONE, B., and BAZETT, H. C. The temperature of the air in contact with the skin, *Am. J. Physiol.*, **82**:452-61, 1927.
291. MEITES, J. Counteraction of cortisone inhibition of body hair and thymus growth by vitamin B₁₂ and aureomycin, *Proc. Soc. Exper. Biol. & Med.*, **78**:692-95, 1951.
292. MENSE, K. Über Hypertrichosis lanuginensis, s. primaria, *Beitr. z. path. Anat.*, **68**:486-95, 1921.
293. MERIVALE, W. H. H. The excretion of pregnanediol and 17-ketosteroids during the menstrual cycle in benign hirsutism, *J. Clin. Path.*, **4**:78-84, 1951.
294. MERZ, W. R. Die Behandlung der Thrombose und Lungenembolie mit Antikoagulantien, *Gynaecologica (suppl.)*, **130**:1-123, 1950.
295. MEYER-LIERHEIM, F. Die Dichtigkeit der Behaarung beim Foetus des Menschen und der Affen, *Ztschr. f. Morphol. u. Anthropol.*, **13**:130-50, 1910.
296. MEYLER, L., and HOMMES, M. The adrenal cortical syndrome, *Acta med. Scandinav.*, **93**:253-64, 1937.
297. MOLT, F. H. Histopathology of rat skin in avitaminosis A, *Arch. Dermat. & Syph.*, **47**:768-77, 1943.
298. MONTAGNA, W., and CHASE, H. B. Redifferentiation of sebaceous glands in the mouse after total extirpation with methylcholanthrene, *Anat. Rec.*, **107**:83-92, 1950.
299. MONTAGNA, W.; CHASE, H. B.; and HAMILTON, J. B. The distribution of glycogen and lipids in human skin, *J. Invest. Dermat.*, **17**:147-57, 1951.
300. MONTGOMERY, D. W. The alopecia of hypothyreosis, *J. Cutan. Dis.*, **33**:260-65, 1915.
301. ———. References to hair by Rabelais, *M. Rec.*, **94**:982-84, 1918.
302. MORRIS, H. P., and LIPPINCOTT, S. W. Effect of pantothenic acid on growth and maintenance of life in C₃H strain of mouse, *J. Biol. Chem.*, **140**:xciii-xciv, 1941.
303. MORRIS, J. P. Zinc content of some tropical foods, *J. Malaya Branch Brit. M. A.*, **4**:114-17, 1940.
304. MORRISSEY, M. J. Influenza alopecia, *J. Cutan. Dis.*, **37**:177-78, 1919.

305. MOTTRAM, J. C. Effect of change of coat on the growth of epidermal warts in mice, *Nature*, **155**:729-30, 1945.
306. MOURICHAUD, G. Épilation et déséquilibre alimentaire, *Compt. rend. Soc. de biol.*, **95**:477-78, 1926.
307. MOXON, A. L., and RHIAN, M. Selenium poisoning, *Physiol. Rev.*, **23**:305-37, 1943.
308. MÜLLER, G. Der erbkonstitutionelle Hypogenitalismus des Mannes als Dispositionsfaktor der Lebercirrhose; zugleich ein Beitrag zur Sexualkonstitution der männlichen Bevölkerung, *Med. Klin*, **47**:71-74, 1952.
309. MUNCH, J. C. Human thallotoxicosis, *J.A.M.A.*, **102**:1929-34, 1934.
310. MURRAY, M. R. Development of the hair follicle and hair in vitro, *Anat. Rec. (suppl.)*, **57**:74, 1933.
311. MUSSIO-FOURNIER, J. C., and CERVINO, J. M. Lanugo e hipotiroidismo, *Arch. clín. e Inst. endocrinol.*, **1**:355-56, 1937-40.
312. MUSSIO-FOURNIER, J. C.; POLLACK, E.; and LUSSICH SIRI, J. J. Loss of axillary and pubic hair in a patient with Addison's disease and regular menstruation. A case report, *J. Clin. Endocrinol.*, **9**:555-56, 1949.
313. MYERS, R. J., and HAMILTON, J. B. Regeneration and rate of growth of hair in man, *Ann. New York Acad. Sc.*, **53**:562-68, 1951.
314. NAIDE, M. Relation of growth of hair on digits to the severity of ischemia, *New England J. Med.*, **248**:179-82, 1953.
315. NAZ, J. F., and EDWARDS, W. M. Hypervitaminosis A. A case report, *New England J. Med.*, **246**:87-89, 1952.
316. NENDA, P. Haarausfall im lateralen Anteil der Augenbraue, *Wien. klin. Wchnschr.*, **41**:482-84, 1928.
317. NEUMANN, F. W.; KRIDER, M. M.; and DAY, H. G. Biotin deficiency in rats fed purified diets containing succinylsulfathiazole and paraaminobenzoic acid, *Proc. Soc. Exper. Biol. & Med.*, **52**:257-60, 1943.
318. NIELSEN, E., and BLACK, A. Role of inositol in alopecia of rats fed sulfasuxidine, *Proc. Soc. Exper. Biol. & Med.*, **55**:14-16, 1944.
319. ———. Biotin and folic acid deficiencies in the mouse, *J. Nutrition*, **28**:203-6, 1944.
320. NIELSEN, E., and ELVEHJEM, C. A. Cure of spectacle eye condition in rats with biotin concentrates, *Proc. Soc. Exper. Biol. & Med.*, **48**:349-52, 1941.
321. NILES, H. D. Relation of the adrenal glands to hypertrichosis. Results of irradiation of the adrenals and review of the literature, *Arch. Dermat. & Syph.*, **32**:580-88, 1935.
322. NISHIMURA, H. Zinc deficiency in suckling mice deprived of colostrum, *J. Nutrition*, **49**:79-98, 1953.
323. NOVAK, L. J., and BERGEIM, O. Water-soluble vitamins in hair as influenced by diet, *J. Biol. Chem.*, **155**:283-90, 1944.
324. NYSTRÖM, A. E. Health hazards in the chloroprene rubber industry and their prevention, *Acta med. Scandinav. (suppl. 219)*, pp. 1-125, 1948.
325. OESCH, F. Versuche mit Pantothenensäure am Menschen, *Schweiz. med. Wchnschr.*, **76**:6-8, 1946.
326. OKAMURA, CH. Die vier Arten der Nervenendapparate der Sinus- und gewöhnlichen Haare der Maus, *Ztschr. f. mikr.-anat. Forsch.*, **42**:578-94, 1937.
327. OKUDA, S. Klinische und experimentelle Untersuchungen über die Transplantation von lebenden Haaren, *Jap. J. Dermat. & Urol.*, **46**:135-38, 1939.
328. OKUDA, Y., and KATAI, K. On the cystine content of hair and feathers, *J. Biochem. (Japan)*, **24**:207-14, 1936.
329. OPPEL, T. W. Studies of biotin metabolism in man. Studies of the mechanism of absorption of biotin and the effect of biotin administration on a few cases of seborrhea and other conditions, *Am. J. M. Sc.*, **215**:76-83, 1948.
330. ORENT-KEILES, E.; ROBINSON, A.; and MCCOLLUM, E. V. The effects of sodium deprivation on the animal organism, *Am. J. Physiol.*, **119**:651-61, 1937.
331. OTTENWÄELDER, A. Recherches botaniques et chimiques sur le *Leucaena glauca* Benth. (mimosées). Thèse. Paris: Vigot Frères, 1939.
332. PAFF, G. H.; SUGIURA, H. T.; BOCHER, C. A.; and ROTH, J. S. The probable mechanism of heparin inhibition of mitosis, *Anat. Rec.*, **114**:499-503, 1952.
333. PAGLIA, A. Sul significato di una particolare alopecia delle gambe negli iperuricemici (alopecia peroniera degli uricemici del Tommasi), *Riforma med.*, **47**:1623-29, 1931.
334. PAINTER, E. P. The chemistry and toxicity

- of selenium compounds with special reference to the selenium problem, *Chem. Rev.*, **28**:179-213, 1941.
335. PANNATTONI, M., and RAGNI, G. L'azione della tetrabromotironina sul trofismo pilifero, *Boll. Soc. ital. biol. sper.*, **26**:1456-57, 1950.
 336. ———. L'azione della tetrabromotironina sul trofismo pilifero, *Arch. di fisiol.*, **50**:322-27, 1951.
 337. PARNELL, J. P. Postnatal development and functional histology of the sebaceous glands in the rat, *Am. J. Anat.*, **85**:41-72, 1949.
 338. PATT, H. M. Protective mechanisms in ionizing radiation injury, *Physiol. Rev.*, **33**:351-76, 1953.
 339. PECK, S. M. Zur Pigmentgenese in der Haut und den Haaren von Kaninchen, *Arch. f. Dermat. u. Syph.*, **157**:234-63, 1929.
 340. PEDERSEN, A. L. Metabolism of androgen in normal-haired and in hypertrichotic schizophrenic women, *Acta psychiat. et neurol. (suppl.)*, **47**:130-40, 1947.
 341. PEDERSEN, J. Studies of menstruation anomalies, fertility and androgen excretion of normally haired and hypertrichotic women, on the diagnosis of hypertrichosis, *Acta dermat.-venereol.*, **24**:49-72, 1943.
 342. ———. The correlation between the degree of hair covering and the excretion of androgenous substances in the urine of normal women, *Ugesk. f. laeger*, **105**:229-34, 1943.
 343. ———. Virilizing ovarian tumors, *J. Clin. Endocrinol.*, **7**:115-29, 1947.
 344. PERUTZ, A. Die Pharmakologie der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **5**/1:1-296. Berlin: J. Springer, 1930.
 345. PETERS, A. D. K. Case of hirsuties treated by ovarian follicular hormone, *Proc. Roy. Soc. Med.*, **27**:809-13, 1934.
 346. PFALTZ, H. Die Vitaminwirkung des γ -Oxypropylamids der 2,4-Dioxy-3,3-dimethylbuttersäure und anderer Abkömmlinge der Pantothersäure, *Ztschr. f. Vitaminforsch.*, **13**:236-49, 1943.
 347. PINKUS, F. Der Ausfall des Kopfhaars und seine Behandlung, *Samml. zwangl. Abh. Dermat.*, **3**:Part I, 1-56, 1914.
 348. ———. Die Einwirkung von Krankheiten auf das Kopfhaar des Menschen. Berlin: S. Karger, 1917.
 349. ———. Zur Kenntnis der Lebensdauer der menschlichen Terminalhaare, *Ztschr. f. Morphol. u. Anthropol.*, **24**:265-69, 1924.
 350. ———. Die normale Anatomie der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1**/1: Berlin: J. Springer, 1927.
 351. ———. The story of a hair root, *J. Invest. Dermat.*, **9**:91-94, 1947.
 352. PINKUS, H. Multiple hairs (Flemming-Giovannini), *J. Invest. Dermat.*, **17**:291-301, 1951.
 353. PLATE, W. P. Hirsutism in ovarian hyperthecosis, *Acta endocrinol.*, **8**:17-32, 1951.
 354. POHL, J. Das Haar. 5th ed. 1902. Quoted by PINKUS, F. (348).
 355. QUACKENBUSH, F. W.; STEENBOCK, H.; KUMMEROW, F. A.; and PLATZ, R. R. Linoleic acid, pyridoxine and pantothenic acid in rat dermatitis, *J. Nutrition*, **24**:225-34, 1942.
 356. REDLICH, E. Über physiologische Hypertrichose. Ein Beitrag zur Kenntnis der Behaarungstypen beim Menschen, *Ztschr. f. Konstitutionslehre*, **12**:740-57, 1926.
 357. RESSMANN, A. C., and BUTTERWORTH, T. Localized acquired hypertrichosis, *Arch. Dermat. & Syph.*, **65**:458-63, 1952.
 358. REYERSBACH, G. C.; HANELIN, J.; and JOPLIN, R. J. Vitamin A intoxication. Report of a case, *New England J. Med.*, **246**:978-80, 1952.
 359. REYNOLDS, S. R. M.; HAMILTON, J. B.; DI PALMA, J. R.; HUBERT, G. R.; and FOSTER, F. I. Dermovascular actions of certain steroid hormones in castrate, eunuchoid and normal men, *J. Clin. Endocrinol.*, **2**:228-36, 1942.
 360. RIDLEY, H. N. The cause of the hairlessness of man, *J. Trop. Med.*, **29**:313-14, 1926.
 361. RIMINGTON, C. The relation between cystine yield and total sulfur in kemp and outer coat animal fibres, *Biochem. J.*, **25**:71-73, 1931.
 362. RITTER, W. L., and CARTER, A. S. Hair loss in neoprene manufacture, *J. Indust. Hyg. & Toxicol.*, **30**:192-95, 1948.
 363. ROBERTS, E.; QUISENBERRY, J. H.; and THOMAS, L. C. Hereditary hypotrichosis in rat, *J. Invest. Dermat.*, **3**:1-29, 1940.
 364. RONCHESE, F. Transitory baldness of a peculiar type following trauma, *Arch. Dermat. & Syph.*, **28**:639-41, 1933.
 365. RONCHESE, F., and CHACE, R. R. Pat-

- terned alopecia about the calves and its apparent lack of significance, Arch. Dermat. & Syph., **40**:416-21, 1939.
366. RONY, H. R., and ZAKON, S. J. Effect of endocrine substances on adult human scalp, Arch. Dermat. & Syph., **52**:323-27, 1945.
 367. ROST, E. Woher stammt das Zink im menschlichen und tierischen Organismus? Med. Klin., **17**:123-24, 1921.
 368. ROTH, E. Ueber behaarte Menschen, Dermat. Zentralbl., **8**:34-40, 1904.
 369. ROTHMAN, S. Die Stickstoffabgabe durch die Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1**/2:226. Berlin: J. Springer, 1929.
 370. ROTHMAN, S., and SCHAAF, F. Chemie der Haut. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1**/2:301-21. Berlin: J. Springer, 1929.
 371. ROYO, F. Determination of zinc in tissues, Trab. Inst. nac. cienc. méd. (Madrid), **5**:239-44, 1944-45.
 372. SAALFELD, E. Periarterielle Histonektomie (Sympathektomie) und Haarwachstum, Arch. f. d. ges. Physiol., **204**:174-76, 1924.
 373. ———. Periarterielle Histonektomie (Sympathektomie) und Haarwachstum, Dermat. Wchnschr., **79**:1257-58, 1924.
 374. SABOURAUD, R. Maladies du cuir chevelu. I. Les maladies séborrhéiques. I. Séborrhée, acnés, calvitie. Paris: Masson et Cie, 1902.
 375. ———. Les alopecies post-fébriles. L'alopecie qui suit la grippe, Paris méd., **31**:437-42, 1919.
 376. SACHS, B., and SPIRO, D. Leydig cell (sympathicotropic) tumor of ovary. Report of a case with virilism, including post-mortem findings, J. Clin. Endocrinol., **11**:878-89, 1951.
 377. SAINTON, P., and SIMMONET, H. Les troubles de la fonction thyroïdienne et leur action sur le système pileux, Ann. de méd., **29**:263-70, 1931.
 378. SAUDEK, J. Ätherspray zur Förderung des Haarwuchses, Dermat. Wchnschr., **85**:1560-62, 1927.
 379. SCHAMBERG, I. L. Studies on atabrine dermatitis. I. Long-term observation of veterans with permanent atrophic residua of the disease, J. Invest. Dermat., **17**:85-98, 1951.
 380. SCHEIN, M. Ueber die Entstehung der Glatze, Wien. med. Wchnschr., **16**:611-15 and 968-70, 1903.
 381. SCHIEFFERDECKER, P. Über die Haarlosigkeit des Menschen. Eine Betrachtung, Anat. Anz., **53**:383-96, 1920.
 382. SCHMIDT, H. E. Einige Versuche betreffend den Einfluss des Lichtes auf das Wachstum der Haare und seine therapeutische Anwendung bei der Alopecia areata, Arch. f. Dermat. u. Syph., **62**:329-38, 1902.
 383. SCHMIDT, V. The excretion of pantothenic acid in patients with achromotrichia and alopecia, J. Gerontol., **6**:369-71, 1951.
 384. SCHÖBERL, A. Grüngefärbte menschliche und tierische Haare, Naturwiss., **34**:217, 1947.
 385. SCHULTZ, A. H. The density of hair in primates, Human Biol., **3**:303-21, 1931.
 386. SCHWANITZ, J. Untersuchungen zur Morphologie und Physiologie des Haarwechsels beim Hauskaninchen, Ztschr. f. Morphol. u. Ökol. Tiere, **33**:496-526, 1938.
 387. SCHWARTZ, J. H. Hirsutism, Psychiat. Quart., **16**:281-94, 1942.
 388. SCHWARTZ, L. Skin hazards in the manufacture and processing of synthetic rubber, J.A.M.A., **127**:389-91, 1945.
 389. SCHWARZ, G. Ueber Desensibilisierung gegen Röntgen- und Radiumstrahlen, München. med. Wchnschr., **56**:1217-18, 1909.
 390. SCHWARZ, L., and DECKERT, W. Studien zur Beurteilung von Arsenbefunden in Ausscheidungen und Hautanhängen, Arch. f. Hyg., **106**:346-65, 1931.
 391. ———. Ueber Arsen in den Haaren und einige andere Befunde bei erkrankten Arsenarbeitern, *ibid.*, **129**:276-85, 1943.
 392. SEBRUYN, M. Recherches histochimiques sur la localisation du zinc dans l'organisme de la souris adulte, Compt. rend. Soc. de biol., **140**:1141-43, 1946.
 393. SEGALL, A. Ueber die Entwicklung und den Wechsel der Haare beim Meerschweinchen (*Cavia cobaya*, Schreb.), Ztschr. f. mikr.-anat. Forsch., **91**:218-91, 1918.
 394. SELLHEIM, H. Vermännlichung und Wiederverweiblichung bei einem ausgewachsenen Individuum, Ztschr. f. mikr.-anat. Forsch., **3**:382-408, 1925.
 395. SEYMOUR, R. J. The effect of cutting upon the rate of hair growth, Am. J. Physiol., **78**:281-86, 1926.

396. SHEPARDSON, H., and SHAPIRO, E. The diabetes of bearded women. Suprarenal tumor diabetes and hirsutism. Clinical correlation of the function of the suprarenal cortex in carbohydrate metabolism, *Endocrinology*, **24**:237-52, 1939.
397. SLEPYAN, A. H. Selenium disulfide suspension in treatment of seborrheic dermatitis of the scalp, *Arch. Dermat. & Syph.*, **65**:228-29, 1952.
398. SLINGER, W. N., and HUBBARD, D. M. Treatment of seborrheic dermatitis with a shampoo containing selenium disulfide, *Arch. Dermat. & Syph.*, **64**:41-48, 1951.
399. SMITH, S., and HENDRY, E. B. Arsenic in its relation to keratin tissue, *Brit. M. J.*, **2**:675-77, 1934.
400. SMITH, S. E., and ELLIS, G. H. Copper deficiency in rabbits. Achromotrichia, alopecia and dermatosis, *Arch. Biochem.*, **15**:81-88, 1947.
401. SMUTS, D. B.; MITCHELL, H. H.; and HAMILTON, T. S. The relation between dietary cystine and the growth and cystine content of hair in the rat, *J. Biol. Chem.*, **95**:283-95, 1932.
402. SNOW, J. S., and WHITEHEAD, R. W. Relationship of hypophysis in albino rat to hair growth, *Endocrinology*, **19**:88-96, 1935.
403. SOKOLOWSKY, A. Das Haarkleid des Menschen in seinen Beziehungen zu dem der Menschenaffen, *Dermat. Wchnschr.*, **88**:432-37, 1929.
404. ———. Das Haarkleid der Säugetiere in biologischer Beziehung, *ibid.*, **96**:373-77, 1933.
405. SPENCER, B., and WILLIAMS, R. T. Studies in detoxication. Influence of bromobenzene and cystine on the bromine content of the hair of rats, *Biochem. J.*, **46**:460-65, 1950.
406. SPIRA, L. Some sources of intake and methods of elimination of fluorine, *Acta med. Scandinav.*, **130**:78-96, 1948.
407. SPITZER, R. R., and PHILLIPS, P. H. Alopecia in rats fed certain soybean oil meal rations, *Proc. Soc. Exper. Biol. & Med.*, **63**:10-13, 1946.
408. SSOBOLEWA, N. G. Die Haarregeneration bei Teermäusen als eins der Konstitutionszeichen, *Ztschr. f. Krebsforsch.*, **37**:224-34, 1932.
409. STARLING, E. H. Principles of human physiology. 9th ed. Philadelphia: Lea & Febiger, 1945.
410. STEIN, I. F.; COHEN, M. R.; and ELSON, R. Results of bilateral ovarian wedge resection in 47 cases of sterility. 20-year end results: 75 cases of bilateral polycystic ovaries, *Am. J. Obst. & Gynec.*, **58**:267-71, 1949.
411. STEIN, I. F., and LEVENTHAL, M. L. Amenorrhea associated with bilateral polycystic ovaries, *Am. J. Obst. & Gynec.*, **29**:181-91, 1935.
412. STEIN, R. O. Untersuchungen über die Ursache der Glatze, *Wien. klin. Wchnschr.*, **37**:6-10, 1924.
413. ———. Die Säuglingsglatze und ihre Beziehung zur Glatze des Erwachsenen, *ibid.*, **46**:729-32 and 1029, 1933.
414. ———. Sui rapporti tra lo sviluppo e la caduta dei peli ed il sistema endocrino; sulle possibilità di una terapia endocrina, *Gazz. d. osp.*, **57**:1169-71, 1936.
415. ———. Ueber die Beziehungen des Haarwachstums und Haarausfalles zum endokrinen System und über die Möglichkeit einer endokrinen Therapie der Glatze, *Wien. klin. Wchnschr.*, **49**:449-52, 1936.
416. STODDARD, F. J. Hirsutism in pregnancy, *Am. J. Obst. & Gynec.*, **49**:417-22, 1945.
417. STOKHUYZEN, A. W. Hirsutisme van ovariele oorsprong? *Nederl. tijdschr. v. verlosk. en gynaec.*, **50**:337-50, 1950.
418. STRANGEWAYS, D. H. The growth of hair in vitro, *Arch. f. exper. Zellforsch.*, **11**:344-45, 1931.
419. STRASSMANN, E. O. Masculine hair growth in women and gynecological treatment, *M. Rec. & Ann.*, **36**:264-68, 1942.
420. STRAUB, W. Ueber chronische Vergiftung, speziell die chronische Bleivergiftung, *Deutsche med. Wchnschr.*, **37**:1469-71, 1911.
421. STRZYZOWSKI, C. The migration of mercury to the "hair substance" after subcutaneous injection of mercury in syphilis. A method for the detection of very small quantities of mercury by means of a microchemical test, *Chem. Ztg.*, **36**:1237-39, 1912.
422. SULLIVAN, M., and NICHOLLS, J. Nutritional dermatoses in the rat. IV. Riboflavin deficiency, *J. Invest. Dermat.*, **4**:181-91, 1941.
423. ———. Nutritional dermatoses in the rat. VI. The effect of pantothenic acid de-

- iciency, Arch. Dermat. & Syph., **45**:917-32, 1942.
424. SULLIVAN, R. D.; MESCON, H.; and JONES, R., JR. The effect of intra-arterial nitrogen-mustard (methylbis(2-chloroethyl) amine hydrochloride) therapy on human skin, Cancer, **6**:288-93, 1953.
425. SULZBERGER, M. B., and LAZAR, M. P. Hypervitaminosis A, J.A.M.A., **146**:788-93, 1951.
426. SUMMERS, V. K. Role of the adrenal cortex and gonads in the control of sexual hair distribution, Acta med. Scandinav., **136**: 105-11, 1949.
427. SYLVÉN, B. The qualitative distribution of metachromatic polysaccharide material during hair growth, Exper. Cell. Research, **1**:582-89, 1950.
428. SZASZ, T. S., and ROBERTSON, A. M. A theory of the pathogenesis of ordinary human baldness, Arch. Dermat. & Syph., **61**:34-48, 1950.
429. SZÉP, Ö. Zur Methodik der Arsenbestimmung in Haaren und Nägeln, Ztschr. f. physiol. Chem., **267**:29-36, 1940.
430. TABOURY, M. M. F. Toxicologie du sélénium et de ses composés, Bull. Soc. chim. biol., **27**:157-63, 1945.
431. TADA, K. Über den Bleigehalt der Kopfhare bei den an "seröse Meningitis" leidenden Säuglingen und ihren Müttern, Orient. J. Dis. Infants, **1**:73-77, 1926.
432. TADOKORO, T., and UGAMI, H. On the cystine and cysteine contents of human hair, J. Biochem., **12**:187-93, 1930.
433. TAMURA, S. A study of alopecia areata. II. A histological study of the slender nerve branches of hair follicles in alopecia areata, Acta dermat., **11**:184, 1928.
434. TEMPLETON, H.; MICHAEL, G.; and KEY, J. M. Epilatory action of ethyl gasoline, Arch. Dermat. & Syph., **37**:35-42, 1938.
435. THIBIERGE, G. L'alopécie post-grippale, Rev. gén. de clin. et de thérap., **33**:1-2, 1919.
436. THIGPEN, L. W. Growth, replacement and types of hair; inheritance-distribution, pattern, quality and quantity, Ann. New York Acad. Sc., **53**:674-81, 1951.
437. THORNER, J. E. Alopecia totalis. With special reference to familial and endocrine factors, Endocrinology, **26**:433-36, 1940.
438. THYRESSON, N. The influence of dietary factors, especially brewer's yeast, cystine and B vitamins, on the course of chronic thallium poisoning in the rat, Acta dermat.-venereol., **30**:9-26, 1950.
439. ———. Experimental investigation of thallium poisoning. Influence of thallium on tissue metabolism, *ibid.*, pp. 417-41.
440. ———. Experimental investigation of thallium poisoning in the rat. Distribution of thallium, especially in the skin, and excretion of thallium under different experimental conditions. A study with the use of the radioactive isotope Tl^{204} , *ibid.*, **31**:3-27, 1951.
441. ———. Experimental investigation of thallium poisoning. Effect of thallium on the growth and differentiation of hairs and epidermis, studied in young rats and in tissue cultures of embryonic rat skin, *ibid.*, pp. 133-46.
442. ———. Effect of thallium on the growth of tactile hair in the white rat, *ibid.* (suppl. 29), **32**:370-75, 1952.
443. TIETZ, E. B. The humoral excitation of the nesting instincts of rabbits, Science, **78**:316, 1933.
444. TIMPANO, P., and CASTORINA, G. L'acetato di tallio nella cura delle tigne e tricofizie, Pediatria, **42**:485-89, 1934.
445. TOMMASI, L. Alopecia of peroneal regions as constitutional signs of neuroarthritic diathesis, Brit. J. Dermat., **52**:1-9, 1940.
446. TRAUTMANN, A. Die Beeinflussung des tierischen Organismus durch phosphatreiche Nahrung; zugleich ein Beitrag zur Resorption der Phosphatide, Tierzücht. u. Züchtungsbiol., **24**:27-41, 1932.
447. TROTTER, M. The resistance of the hair to certain supposed growth stimulants, Arch. Dermat. & Syph., **7**:91-98, 1923.
448. ———. The life cycle of hair in selected regions of the body, Am. J. Phys. Anthropol., **7**:427-37, 1924.
449. ———. Hair growth and shaving, Anat. Rec., **37**:373-79, 1928.
450. ———. The hair. In: COWDRY, E. V., Special cytology, **1**:41-65. New York: P. B. Hoeber, 1932.
451. ———. The activity of hair follicles with reference to pregnancy, Surg., Gynec. & Obst., **60**:1092-95, 1935.
452. TROTTER, M., and DUGGINS, O. H. Age changes in head hair from birth to maturity. I. Index and size of hair, Am. J. Phys. Anthropol., **6**:489-506, 1948.
453. ———. Age changes in head hair from birth to maturity. III. Cuticular scale

- counts of hair of children, *ibid.*, **8**:467-84, 1950.
454. TRUFFI, G. Sulla alopecia da tallio. II. Meccanismo d'azione del tallio nella produzione della alopecia nel bambino, *Gior. ital. di dermat. e sif.*, **69**:60-80, 1928.
 455. ———. Azione biologica dell'acetato di tallio, *Arch. di sc. biol.*, **13**:271-319, 1929.
 456. ———. Sull'azione biologica dell'acetato di tallio, *Gior. ital. di dermat. e sif.*, **70**:872-79, 1929.
 457. TSCHERNJACHOWSKY, A. Zur Frage über die Nervenendigungen des Haares (Terminaisons foraminaux de Tello), *Anat. Anz.*, **75**:169-74, 1932.
 458. TSCHERNOGUBOW, N. A. Zur Frage nach dem Mechanismus der Heilwirkung der X-Strahlen bei Pilzkrankungen der behaarten Kopfhaut und nach der Bedeutung der medikamentösen Lokalbehandlung dabei, *Dermat. Wchnschr.*, **82**:5-8, 1926.
 459. VAN DYKE, D. C., and HUFF, R. L. Epilation in the non-irradiated member of parabiotically united rats, *Proc. Soc. Exper. Biol. & Med.*, **72**:266-69, 1942.
 460. VERZÁR, F., and KOKAS, E. VON. Die Wirkung des Mangels an E-Vitamin auf das Haarkleid der Ratten. XIII, *Arch. f. d. ges. Physiol.*, **227**:511-16, 1931.
 461. VIGNOLO-LUTATI, K. Ueber die experimentelle Alopecien durch Abrin, *Arch. f. Dermat. u. Syph.*, **111**:549-60, 1912.
 462. VITTE, G. Quelques dosages d'arsenic dans les cheveux au cours d'intoxication chronique, *Ann. de méd. lég.*, **28**:164-65, 1948.
 463. ———. The bromine content of hair, *Bull. trav. soc. pharm. Bordeaux*, **78**:69-70, 1940.
 464. VOIT, E. Ueber die Grösse der Erneuerung der Horngelbe beim Menschen. I. Die Haare, *Ztschr. f. Biol.*, **90**:509-24, 1930.
 465. VORHAUS, M. G., and GOMPERTZ, M. L. Clinical experiments with riboflavin, inositol and calcium pantothenate, *Am. J. Digest. Dis.*, **10**:45-48, 1943.
 466. WADEL, J. Untersuchungen über den prämaturen chronischen Haarausfall, *Wien. klin. Wchnschr.*, **46**:1383-85, 1933.
 467. ———. Neue Untersuchungen über die Entstehung der prämaturen Alopecie, *Arch. f. Dermat. u. Syph.*, **172**:128-31, 1935.
 468. WALD, G., and BROWN, P. K. The role of sulfhydryl groups in the bleaching and synthesis of rhodopsin, *J. Gen. Physiol.*, **35**:797-821, 1951.
 469. WALKER, S. A., and ROTHMAN, S. Statistical study and consideration of endocrine influences in alopecia areata, *J. Invest. Dermat.*, **14**:403-13, 1950.
 470. WALLENSTERN, L. VON. Ueber die Beeinflussung des Haarwuchses durch experimentelle Wunden am Kaninchen, *Dermat. Wchnschr.*, **122**:831-34, 1950.
 471. WATROUS, R. M., and McCAUGHEY, M. B. Occupational exposure to arsenic in the manufacture of arspenamine and related compounds, *Indust. Med.*, **14**:639-46, 1945.
 472. WEIL, K., and KUHN, W. Untersuchungen über den optischen Reinheitsgrad von Aminosäuren in natürlichen Eiweissstoffen, *Helvet. chim. acta*, **27**:1648-69, 1944.
 473. WESTFALL, B. B., and SMITH, M. I. Selenium in the hair as an index of the extent of its deposition in the tissues in chronic poisoning, *Nat. Inst. Health Bull.*, **174**:45-49, 1940.
 474. WHITAKER, W. L. The stimulation of human hair production by the topical application of testosterone, *Univ. Hosp. Bull., Ann Arbor*, **8**:46-47, 1942.
 475. WHITAKER, W. L., and BAKER, B. L. Inhibition of hair growth by the percutaneous application of certain adrenal cortical preparations, *Science*, **108**:207-9, 1948.
 476. ———. A comparison of the direct action of estrogen and adrenal cortical extracts on growth of hair in the rat, *J. Invest. Dermat.*, **17**:69-77, 1951.
 477. WHITELEY, H. J., and GHADIALLY, F. N. The naturally occurring effect of zones of hair growth on experimental carcinogenesis in the rabbit, *Brit. J. Cancer*, **5**:353-57, 1951.
 478. WILKINS, L.; CRIGLER, J. F.; SILVERMAN, S. H.; GARDNER, L. I.; and MIGEON, C. J. Further studies on the treatment of congenital adrenal hyperplasia with cortisone. II. The effect of cortisone on sexual and somatic development with an hypothesis concerning the mechanism of feminization, *J. Clin. Endocrinol.*, **12**:277-95, 1952.
 479. WILKINS, L.; GARDNER, L. I.; CRIGLER, J. F.; SILVERMAN, S. H.; and MIGEON, C. J. Further studies on the treatment of congenital adrenal hyperplasia with cortisone. I. Comparison of oral and intramuscular administration of cortisone with

- a note on the suppressive action of compounds F and B on the adrenal, *J. Clin. Endocrinol.*, **12**:257-76, 1952.
480. WILKINS, L.; LEWIS, R. A.; KLEIN, R.; GARDNER, L. I.; CRIGLER, J. F., JR.; ROSEMBERG, E.; and MIGEON, C. J. Treatment of congenital adrenal hyperplasia with cortisone, *J. Clin. Endocrinol.*, **11**:1-25, 1951.
481. WILLIAMS, A. W. A note on certain appearances of X-rayed hairs, *Brit. J. Dermat.*, **18**:63-65, 1906.
482. WILLIAMS, R. H. Thyroid and adrenal interrelations with special reference to hypotrichosis axillaris in thyrotoxicosis, *J. Clin. Endocrinol.*, **7**:52-57, 1947.
483. WILSON, R. A. Effect of ACTH on hair growth in alopecia areata and universalis, *Lancet*, **262**:646-47, 1952.
484. WINKLER, W. Ein Fall von Syringomyelie mit einseitiger segmentaler Alopecie, *Wien. klin. Wchnschr.*, **43**:1113-16, 1930.
485. WINSAUER, H. J., and MANNING, J. C. A masculinizing tumor of the ovary in a postmenopausal woman, *J. Clin. Endocrinol.*, **9**:774-81, 1949.
486. WINTER, H. Wird das Wachstum der Haare, insbesondere das Wachstum des Frauenbartes durch Rasieren beeinflusst? *Dermat. Wchnschr.*, **121**:73-81, 1950.
487. WINTROBE, M. M.; FOLLIS, R. H.; AL-CAYAGA, R.; PAULSON, M.; and HUMPHREYS, S. Pantothenic acid deficiency in swine. With particular reference to the effects on growth and on the alimentary tract, *Bull. Johns Hopkins Hosp.*, **73**:313-41, 1943.
488. WOLBACH, S. B. The hair cycle of the mouse and its importance in the study of sequences of experimental carcinogenesis, *Ann. New York Acad. Sc.*, **53**:517-36, 1951.
489. WOOLLEY, D. W. A new dietary essential for the mouse, *J. Biol. Chem.*, **136**:113-18, 1940.
490. ———. The nature of the antialopecia factor, *Science*, **92**:384-85, 1940.
491. ———. Identification of the mouse antialopecia factor, *J. Biol. Chem.*, **139**:29-34, 1941.
492. ———. Relationship of pantothenic acid and inositol to alopecia in mice, *Proc. Soc. Exper. Biol. & Med.*, **46**:565-69, 1941.
493. WRIGHT, C. S., and HARKINS, M. J. Etiology of alopecia areata with special reference to the effect of experimental nerve injuries, *Arch. Dermat. & Syph.*, **19**:365-77, 1929.
494. WÜHRER, J. Ueber den normalen Arsengehalt des menschlichen Haares, *Biochem. Ztschr.*, **294**:401-6, 1937.
495. YOSIKAWA, H. Studies on biochemistry of copper. XVI. Copper in black and white hairs of aged people. XX. Copper in keratinous appendages of skin, *Jap. J. M. Sc.*, Part II, *Biochemistry*, **3**:195-96 and 269-72, 1937.
496. YOUNG, E. G., and RICE, F. A. H. On the occurrence of arsenic in human hair and its medicolegal significance, *J. Lab. & Clin. Med.*, **29**:439-46, 1944.
497. YOUNG, E. G., and SMITH, R. P. The arsenic content of hair and bone in acute and chronic arsenical poisoning. Review of two cases examined posthumously from a medico-legal aspect, *Brit. M. J.*, **1**:251-53, 1942.
498. YOUNG, M. W. Anatomical factors in senile alopecia; experimental production of baldness, *Anat. Rec.*, **97**:378, 1947.
499. ZIMMERMANN, K. W. Ueber einige Formverhältnisse der Haarfollikel des Menschen, *Ztschr. f. mikr.-anat. Forsch.*, **38**:503-53, 1935.
500. ZUNTZ, N. Beeinflussung des Wachstums der Horngebilde (Haare, Nägel, Epidermis) durch spezifische Ernährung, *Deutsche med. Wchnschr.*, **46**:145-46, 1920.
501. ZURHELLE, E. Zur Kenntnis der Alopecia diffusa nach Grippe, *Deutsche med. Wchnschr.*, **45**:543-45, 1919.

CHAPTER 27

Nutritional Influences

By ALLAN L. LORINCZ¹

I. INTRODUCTION	662
II. GENERAL OVEREATING	663
III. GENERAL UNDERNUTRITION	663
IV. SPECIFIC NUTRITIONAL FACTORS IN MALNUTRITION	663
A. Amino Acids and Proteins	663
B. Vitamins	665
1. B-Factors	665
a) Thiamine	665
b) Riboflavin	665
c) Nicotinic Acid	668
d) Vitamin B ₆	669
e) Pantothenic Acid	672
f) Biotin	673
g) Inositol	675
h) Para-aminobenzoic Acid	676
i) Folic Acid	676
j) Cyanocobalamin	677
k) Amounts Present in Skin	677
2. Ascorbic Acid	677
3. Vitamin P	679
4. Vitamin A	679
5. Vitamin D	681
6. Vitamin E	683
7. Vitamin K	683
C. Essential Fatty Acids	684
D. Minerals and Trace Elements	685
1. Calcium	686
2. Magnesium	686
3. Zinc	688
4. Copper	688

✓ I. INTRODUCTION

OBSERVATIONS of nutritional deficiencies produced experimentally in animals and man have made it obvious that for the maintenance of normal functions of the

1. The author is indebted to Dr. Bernard S. Schweigert for his critical reading of the manuscript of this chapter.

tegument a diet is required which is not deficient in essential nutritional factors. In many of the experimental deficiencies, severe cutaneous disorders are conspicuous and early signs of the deficiency syndrome. With a few exceptions, however, these cutaneous disorders are not characteristic

enough to allow us to draw conclusions about the influence of single factors on single physiological functions. Clinical experience has taught that responses of the skin to widely varying stimuli may be identical, and specific responses cannot always be ex-

pected. In addition, a complicating factor arises from the interrelationship of nutritional factors. This circumstance may cause withdrawal of a single factor to lead to disturbances in the utilization of others.

II. GENERAL OVEREATING

General overeating leads to alimentary obesity. This predisposes to intertrigo dermatitis of many sorts secondary to heat and moisture accumulation between folds. These conditions favor the growth of potentially pathogenic bacteria and fungi in these areas. Excessive stretching of the skin in obesity in predisposed individuals also causes striae distensae.

Dissipation of body heat by conduction

and radiation is also impaired by the thick subcutaneous adipose layer in obese individuals, and, in general, they become easily overheated (12). As a consequence, sweating tends to be more profuse.

The stimulating effect of high caloric intake, especially in the form of fats and carbohydrates, on depot fat in the corium and on sebaceous-gland activity has already been discussed (pp. 483 and 302).

III. GENERAL UNDERNUTRITION

In general undernourishment, whether from food deprivation or in association with severe gastrointestinal diseases, the skin becomes pale, dry, and less elastic. In addition, scaling, disturbances of melanin formation, hemorrhagic tendencies, and increased susceptibility to many kinds of irritations and infections may become manifest. Nutritional edema and dystrophic changes in the nails and hair may also develop. In general, a complex mosaic of the cutaneous mani-

festations that will be discussed under the various specific deficiency syndromes usually occurs. The states of undernourishment, whether general or specific, can come about through several mechanisms: (1) interference with intake, (2) interference with absorption, (3) interference with utilization or storage, (4) increased requirements, (5) increased excretion, and (6) in specific undernutrition through inhibition by "antistances."

IV. SPECIFIC NUTRITIONAL FACTORS IN MALNUTRITION

The nutritional factors involved in specific under- and overnutrition and their associated cutaneous disturbances will be considered under four headings: (1) amino acids and proteins, (2) vitamins, (3) fatty acids, and (4) minerals and trace elements.

A. AMINO ACIDS AND PROTEINS

Deficiency of protein or essential amino acid intake leads to a negative nitrogen balance, which, if sufficiently prolonged, results in a drop in plasma protein concentration before other more serious manifestations become evident. This decrease in plasma proteins, if of sufficient degree, leads

to a generalized edema which is early reflected in the skin. The decreased concentration of plasma protein first develops in the albumin fraction. As it is this fraction which is primarily responsible for maintaining the colloid osmotic pressure of the blood, when it falls below 2.5 gm. per cent, there is no longer sufficient colloid osmotic pressure within the blood vessels to counterbalance the passage of fluid out of them into the tissues from blood pressure filtration, and hence edema develops (p. 72). Synonyms for this protein-deficiency edema are "hunger edema," "war edema," "prison edema," and "alimentary dropsy." During famines

and wars such protein-deficiency edemas have afflicted large masses of the population. Associated with protein-deficiency edema there is a striking drop in resistance to infections of many sorts. This has been related to inadequate ability to form antibodies (28).

Other causes of negative nitrogen balance, besides inadequate dietary intake of essential amino acids, can lead to hypoproteinemic edema. Nephrosis, severe liver disease, and gastrointestinal diseases associated with faulty absorption of food materials are some common examples of such other causes.

In normal adult men, only eight of the amino acids have been shown to be essential for maintaining positive nitrogen balance over a 2-month period (183). These are tryptophane, lysine, phenylalanine, leucine, isoleucine, threonine, methionine, and valine. Tissue requirements for other amino acids can apparently be met by metabolic synthesis. The deficiency of any one of the essential amino acids alone can prevent protein synthesis (71).

Cutaneous effects of amino acid deficiencies, aside from protein-deficiency edema, are difficult to find and prove. However, rats on such deficient diets show thinning of the epidermis, are prone to develop skin infections, and lose hair. The latter in part might be the result of hair-eating, because trichobezoars are not infrequently found in the stomachs of such animals. Methionine- and cystine-deficient rats show definitely inhibited hair growth and faulty hair structure (207, 94). Tryptophane-deficient rats also show hair loss prominently (2).

The possible role of phenylalanine deficiency in preventing normal melanin formation was considered on page 542, as was the suggestion that individuals suffering from phenylpyruvic oligophrenia show deficient melanin formation because of their inability to synthesize tyrosine from phenylalanine. It has also been conspicuously noted that these individuals with phenylketonuria are particularly prone to develop "eczematous" skin lesions (108), which

again might possibly be linked with tyrosine deficiency.

In animals, the finding that a deficiency of sulfur-containing amino acids leads to defective hair growth and appearance is to be expected, in view of the important role of the sulfur-containing amino acids in keratin formation. It is, however, conspicuous that in cystinuria (3), where large amounts of cystine are excreted and lost via the urine, no disturbance of keratinization has been noted. On the other hand, individuals with this metabolic defect can utilize cystine, because dietary administration of cystine does not increase cystine loss in the urine. Faulty methionine metabolism is probably the source of the urinary cystine in this disease.

Recently a curious phenomenon has been observed in studies on the toxicity of vanadium in rats. It has been found that the toxicity syndrome induced by this element is characterized early by defective hair growth and appearance, which exactly duplicates that noted in methionine or cystine deficiency.² It has been postulated that vanadium interferes with the conversion of methionine to cystine. If this postulate is true, vanadium might be useful therapeutically in cystinuria. These new observations also suggest that normal hair formation requires conversion of methionine into cystine, a postulate mentioned on page 368 in connection with the nature of keratinization.

In alkaptonuria (171), another disease involving defective amino acid metabolism, one pathway of tyrosine metabolism is blocked near its end, so that homogentisic acid cannot be converted to acetoacetic acid. Homogentisic acid therefore accumulates and is excreted in the urine, where it turns to a black pigment on oxidation. A similar pigment becomes deposited in various tissues of some such individuals who develop ochronosis. This pigment is deposited in the form of loose clumps in the

2. John T. Mountain, Laryl Lee Delker, and H. E. Stokinger, Studies in vanadium toxicology, *Arch. Indust. Hyg. & Occupational Med.*, **8**:406-11, 1953.

corium of the skin and in cartilages and tendons.

Alkaptonuria has been produced in premature infants (136) and guinea pigs (195) by simultaneous vitamin C deficiency and tyrosine or phenylalanine feeding. Supplementation of the diet with folic acid corrects this abnormality (162), indicating an inter-relationship of vitamin C and folic acid in metabolism.

B. VITAMINS

1. B-Factors

Studies on the mixture of water-soluble vitamin factors in liver and yeast constitute one of the most intricate and exciting chapters in the field of nutrition. Not only have there been described many specific deficiency syndromes which often involve cutaneous disturbances, but also the specific biochemical roles of many of these vitamins have been discovered.

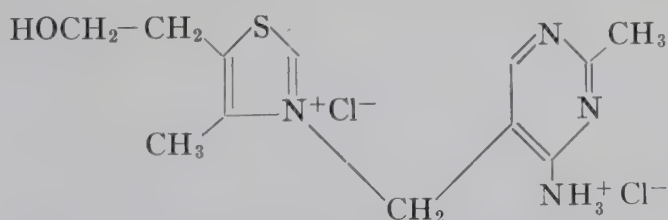
a) Thiamine.—Thiamine (vitamin B₁), the heat-labile factor of the B complex, contains a pyrimidine and a thiazole nucleus (69, 34). In animal tissues in the form of diphosphothiamine, it is cocarboxylase (140), a most important coenzyme in carbohydrate metabolism. This coenzyme functions to bring about the oxidative decarboxylation

in favor of Barron's contention that carbohydrate metabolism in the skin does not go by way of the Krebs tricarboxylic acid or citric acid cycle (11). Edema secondary to cardiac failure, of course, is reflected in the skin in severe thiamine deficiency.

Thiamine, when administered alone to pellagrous individuals, whose disease originates chiefly from lack of nicotinic acid, another member of the B complex, dangerously exacerbates this syndrome and may even precipitate death.

Pyriethamine, a chemical analogue of thiamine with a pyridine ring substituted for the thiazole ring, acts as a metabolic antagonist of thiamine and can produce the thiamine-deficiency syndrome. It is a very potent inhibitor of some dermatophytes (59).

b) Riboflavin.—Riboflavin (vitamin B₂) is composed of 6,7-dimethylisoalloxazine and a ribityl group (129, 130), the former nucleus being responsible for its yellow color and fluorescent properties. In the intestine it is phosphorylated to riboflavin-5-phosphate (cytoflavin), which then functions as such or combined with adenylic acid in the form of riboflavin-adenine-dinucleotide as the prosthetic group of a number of flavo-protein yellow enzymes (239) with key



Structural formula of thiamine hydrochloride

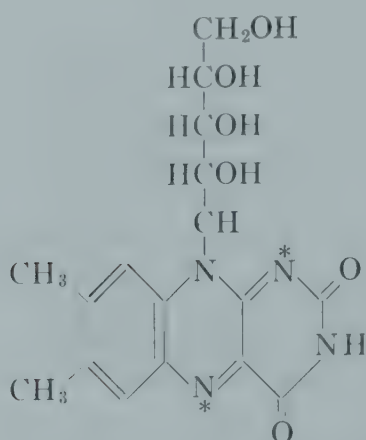
of α -keto acids, such as pyruvic and α -ketoglutaric acids. Its mechanism of action is closely linked in these reactions with coenzyme A and α -lipoic acid.³

A deficiency of thiamine causes the accumulation of pyruvic and lactic acids in the tissues (174, 236) and the development of the disease syndrome, beriberi (146, 243). Curiously, direct cutaneous effects of this deficiency are lacking; and this might argue

functions as hydrogen carriers between the pyridine nucleotides and the cytochrome system, the hydrogen being added to the

3. L. J. Reed and B. G. DeBusk, Lipothiamide and its relation to a thiamine coenzyme required for oxidative decarboxylation of α -keto acids, *J. Am. Chem. Soc.*, **74**:3457, 1952; also Lipothiamide pyrophosphate: coenzyme for oxidative decarboxylation of α -keto acids, *ibid.*, pp. 3964-65; and Lipoic acid conjugase, *ibid.*, pp. 4727-28.

isoalloxazine structure at the points marked by asterisks as illustrated below:



Structural formula of riboflavin

Either isoriboflavin (the 5,6-dimethyl isomer), or the phenazine analogue of riboflavin, can inhibit riboflavin action in rats and mice by metabolic competition (57). It has also been suggested that Atabrine may be a metabolic riboflavin antagonist.

Riboflavin deficiency causes growth failure and prominent changes, especially in the skin (Fig. 1), mucous membranes, eyes, and nervous systems of several species of mammals, including man. In the rat (219) the fur becomes uneven, ragged, and crusted with a dark red-brown material. Partial alopecia occurs over the abdomen, and this area shows a scaly condition. The eyelids and lips also become bald, and the latter become red and swollen. Microscopically, atrophy of the epidermis, follicles, sweat glands, and especially the sebaceous glands is found. It has been suggested that this atrophy of the epidermal structures results from defective hepatic inactivation of estrogen (60), because liver slices from animals depleted in riboflavin fail to inactivate estradiol as do normal liver slices (202), and large amounts of estrogen can cause similar epidermal atrophy (99). Similar general cutaneous changes have been reported in riboflavin-deficient swine (248) and monkeys (235).

In the eye, corneal vascularization from the periphery is the chief manifestation of

this deficiency syndrome. It has been suggested that this vascularization (60) represents an attempt to increase the supply of riboflavin to the cornea, which ordinarily receives its supply of this vitamin from the lacrimal and Meibomian gland secretions, which normally contain very large amounts of this vitamin and early reflect dietary deficiency (175). The picture of experimental riboflavin insufficiency in some animals also includes faulty growth of horns and hoofs (248) and graying of hair (106).

In man, since the work of Sebrell and Butler (196), a syndrome characterized by cheilosis, sore magenta tongue, seborrheic dermatitis about the nose and scrotum, and vascularization of the cornea has been generally accepted as the result of riboflavin deficiency. However, Horwitt *et al.* (100) recently reviewed the many contradictory reports on the subject and in carefully conducted human experiments of their own, involving pure riboflavin deficiency, pointed out that all features of the syndrome do not necessarily develop in all cases. They suggest that the types of abnormalities observed depend upon the triggering action of local trauma, irritation, or infection and that the vitamin deficiency simply prevents normal healing. In deficient rats they actually observed grossly defective healing of experimental wounds. In man, the so-called "characteristic" skin lesions of the deficiency were often noted to develop only after some type of local irritative trigger, e.g., herpes simplex at the corner of the mouth, nasal irritation after a cold, etc. Skin lesions were observed especially often in red-haired, fair-skinned individuals. Corneal vascularization was only rarely observed, in contrast to the high incidence reported among aviators exposed to glare and eyestrain (227).

In man, rosacea keratitis very closely resembles the ocular changes attributed to riboflavin deficiency, and beneficial effects have sometimes followed riboflavin administration (36, 152). It might be of interest to test the effect of local applications of riboflavin-adenine-dinucleotide or cytoflavin on the eyes of individuals with rosacea

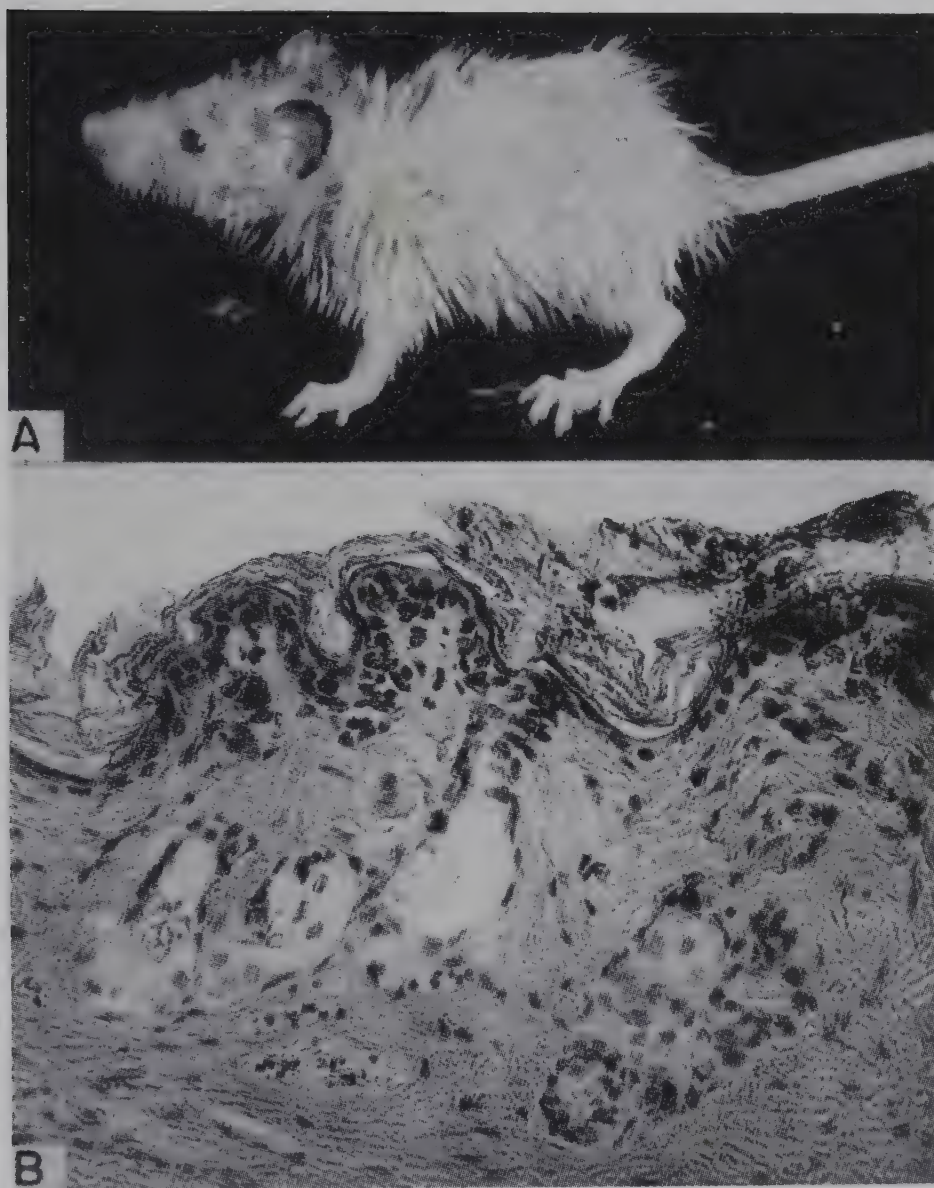
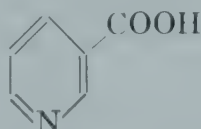


FIG. 1.—*A*, rat which had been on a riboflavin-deficient diet for about 6 weeks. Note uneven and ragged appearance of the fur and stunting of the animal. Alopecia usually begins at this stage. *B*, section of skin shows disintegration of sebaceous-gland cells, with loss of nuclei. This change occurs a little later. In addition, there is beginning atrophy of the epithelium, with slight hyperkeratosis. From Follis (60). (Reproduced by permission of Dr. Follis and Charles C Thomas.)

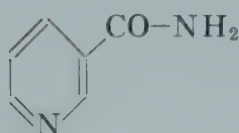
keratitis of the type unresponsive to systemic riboflavin administration.

In general, in man, the mucocutaneous junctions are more vulnerable and the skin less vulnerable to riboflavin deficiency than is the case in laboratory animals (185).

c) *Nicotinic acid*.—Nicotinic acid (niacin) is pyridine-3-carboxylic acid:



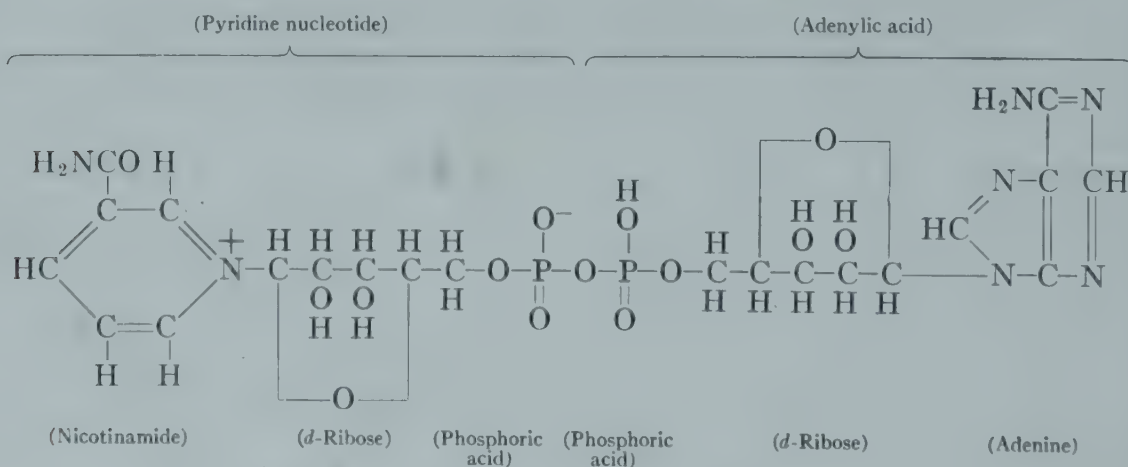
Nicotinic acid
(niacin)



Nicotinamide
(niacinamide)

Structural formulae of nicotinic acid
and nicotinamide

In the body it is transformed into the amide, which then enters into the formation of coenzyme I (cozymase I, diphosphopyridine nucleotide, DPN) and coenzyme II (cozymase II, triphosphopyridine nucleotide, TPN)—two very important hydrogen carriers in cellular respiration (55, 240). Both of these are heat-stable, dialyzable organic substances which are destroyed by ultra-violet radiation. The structure of coenzyme I is illustrated below:



Cozymase I or diphosphopyridine nucleotide

Reduced cozymases are oxidized by orthoquinones but not by molecular oxygen. They ordinarily transfer their hydrogen to the flavoprotein enzymes. Nucleosidases, released from cell nuclei by injury, dephosphorylate and thus inactivate coenzymes I and II. Nicotinamide, however, can inhibit these nucleosidases.

In animals, nicotinuric acid and the salts and esters of nicotinic acid, as well as nicotinamide and coramine, can be utilized for vitamin activity.

Chittenden and Underhill (33) in England and Goldberger (241) and others in this country described a dietary deficiency syndrome in dogs characterized by anorexia, fetid pustular and shreddy mouth and tongue lesions, bloody diarrhea, prostration, and death, and they pointed out pathological similarities to human pellagra. This canine syndrome was called "blacktongue" in America. Goldberger then, with his classical corn-meal prison diet, was able to produce pellagra in man and blacktongue in dogs (73).

The skin lesions of pellagra tend to occur symmetrically on the face (butterfly area), backs of hands, forearms, and dorsa of feet. They favor areas exposed to sunlight, heat, pressure, friction, or other irritating factors tending to interfere with normal cutaneous metabolism. The early acute lesions are sharply outlined and bright red, resembling sunburn. Bullae, with separation

at the epidermal-dermal junction, crusting, and desquamation, may follow, after which there is a dusky reddish-brown pigmentation. In man, porphyrinuria in the past was believed to be associated with the syndrome; but more recent evidence indicates that the pigment excreted in the urine is urochrome or some other indole derivative rather than porphyrin (75). The mechanism of the apparent light sensitivity is unknown. Chronic lesions appear as sharply outlined, thickened, indurated, heavily scale-covered, pigmented areas. Hyperkeratotic follicular plugging is also frequently present, especially on the face. Atrophy of the epidermis may follow chronic lesions.

The mouth and tongue are early affected, at first with slight edema and a burning sensation. Later fiery, scarlet-red inflammation occurs, with much swelling. The edematous tongue often becomes indented around its edges by the teeth, and its papillae, which initially were engorged, become atrophic. Ulcers eventually appear which resemble those of acute aphthous stomatitis. Perianal lesions also commonly occur.

In 1937 Elvehjem (51) demonstrated that nicotinic acid cured the blacktongue syndrome in dogs, and it was soon found that nicotinic acid, likewise, has a strikingly beneficial effect on human pellagra. For a time thereafter it was generally believed that both blacktongue and pellagra were caused specifically by a dietary deficiency of nicotinic acid. More recent observations, however, have complicated this picture. Thus no significant differences in cozymase content of normal and blacktongue-syndrome dog tissues were found (43). Furthermore, diets with a very low content of nicotinic acid alone did not cause the blacktongue syndrome (87) or pellagra. Also, the amino acid tryptophane was found to be an effective substitute for nicotinic acid in rats deficient in this vitamin (126). It was then shown that methylnicotinamide excretion in the urine increases after tryptophane administration (184), which suggested that nicotinic acid may be formed

from dietary tryptophane. Later it was definitely established with radioactive carbon tracers that this conversion actually occurs in mammals (95).

To complicate the picture even more, Woolley (258) has isolated a "pellagrogenic" agent from corn which seems to be a naturally occurring pyridine-containing metabolic antagonist of nicotinic acid. Recent experiments in man by Horwitt and co-workers also lend support to this latter concept of a "pellagrogenic" agent in corn, because pellagra could not be produced on noncorn-containing diets which contained no more nicotinic acid or tryptophane than the original Goldberger prison diet.⁴

Thus it appears that several dietary factors are involved in pellagra. These are (1) low nicotinic acid content, (2) protein of poor quality (low tryptophane content), and (3) the presence of an antinicotinic substance or toxic factor.

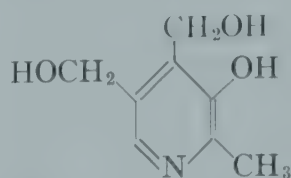
The pharmacologic vasodilatory action of nicotinic acid was discussed on page 104. The normal adult human dietary requirement of nicotinic acid is around 20 mg. daily. The intestinal flora supplies, in addition, a large fraction of the human requirement of this vitamin. Several metabolically competitive analogues of nicotinic acid are known. Medically important competitors are sulfapyridine and isonicotinic acid hydrazide. The latter has been reported to precipitate, on occasion, a pellagra-like syndrome in man (148).

Therapeutically, attempts have been made to use nicotinic acid in a variety of skin disorders, with variably reported success. Its most consistent beneficial effect, if pellagra is excluded, is perhaps in the treatment of dermatitis herpetiformis, where the pyridine structure in general seems to have therapeutic value (109).

d) *Vitamin B₆* (*pyridoxine*, *pyridoxal*, *pyridoxamine*).—Pyridoxine, another member of the heat-stable fraction of the vitamin

4. M. K. Horwitt, personal communication.

B complex, has the following structure (82, 83, 14, 120, 213, 91, 86):



Structural formula of pyridoxine

It is inactivated by light. Biochemically, pyridoxine functions in the form of pyridoxal phosphate (124), which serves as a coenzyme for amino acid decarboxylating and transaminating enzymes. Furthermore, vitamin B₆ plays an important role in tryptophane metabolism (133, 134, 180, 249, 29).

A relationship between vitamin B₆ and essential fatty acids in the rat has also been observed by several workers (13, 177, 191, 188). Finally, Gross (78) showed that pyridoxine is effective in curing "rat acrodynia," a syndrome produced in the rat by either pyridoxine or essential fatty-acid deficiency, only if the animals are also given essential fatty acids. The pyridoxine under these conditions has an essential fatty-acid-sparing action. It seems likely that vitamin B₆ functions in the biological synthesis of fats from protein.

Vitamin B₆ deficiency in the rat causes most conspicuous injury to the skin (Fig. 2) (218). Initially, there suddenly appears erythema of the dorsa of the paws, at first most commonly involving the hind ones. This spreads to the plantar aspects of the feet and is followed by hyperkeratosis, scaling, and edema. A similar process appears on the ears, nose, chin, and upper thorax. There is little alopecia until very late in the deficiency. Histologically, there is hyperkeratosis, acanthosis, and edema in the corium. Late in the syndrome there is atrophy of the cutaneous appendages, which has been thought to be either primary (252, 10) or secondary to infection following ulceration (218).

Cold hastens the development of these lesions. Exposure to light has no effect on the deficiency syndrome. A high-protein diet

also hastens the appearance of the syndrome, whereas a low-protein diet delays it.

The development and growth of some experimental tumors in mice and rats has been inhibited by pyridoxine deficiency (155, 15).

In adult man, vitamin B₆ deficiency has been a much-debated subject, although the vitamin has been a useful adjunct in the treatment of residual manifestations of other vitamin B-deficiency states, like pellagra (208, 209, 261) and some types of cheilosis (204). In infants a convulsive syndrome and anemia have been related to pyridoxine deficiency.⁵

A relationship between seborrheic dermatitis of man and rat acrodynia was early suggested by György (85), and later workers claimed varying therapeutic success in the treatment of seborrheic dermatitis (79, 261, 6) with pyridoxine, as well as in the treatment of acne vulgaris (110).

Most recently, Schreiner *et al.* (193) have noted in patients given 4-desoxypyridoxine, a metabolic antagonist of pyridoxine, the development of a dermatitis resembling seborrheic dermatitis of the sicca type. They further found that pyridoxine, given internally or topically applied, specifically corrected these lesions. They also claim variable success in the treatment of ordinary seborrheic dermatitis, particularly in elderly individuals with lesions about the hairline, with an ointment containing 10 mg. of pyridoxine per gram. They suggest that seborrheic dermatitis may represent a local metabolic defect in the skin involving pyridoxine. The present writer, however, has been unable to observe any specific beneficial effects of pyridoxine ointment, such as described, in a number of individuals with seborrheic dermatitis. Possibly if the preparations contained pyridoxal, the biologically

5. S. E. Snyderman, L. E. Holt, R. Carretero, and K. Jacobs, Pyridoxine deficiency in the human infant, *J. Clin. Nutrition*, 1:200-207, 1953. See also C. J. Molony and A. H. Parmelee, Convulsions in young infants as a result of pyridoxine (vitamin B₆) deficiency, *J.A.M.A.*, 154:405-6, 1954; and D. B. Coursin, Convulsive seizures in infants with pyridoxine-deficient diet, *J.A.M.A.*, 154:406-8, 1954.

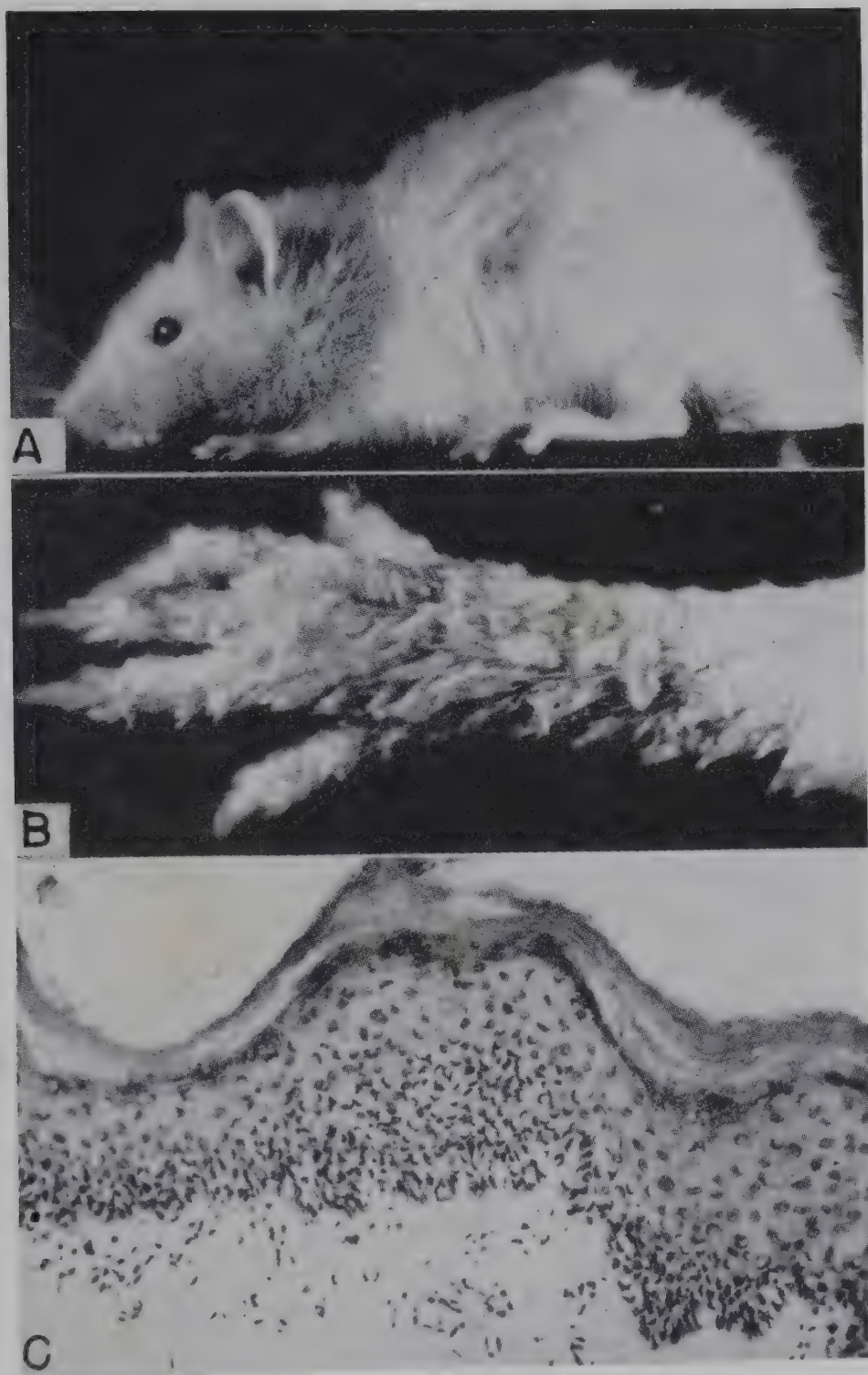
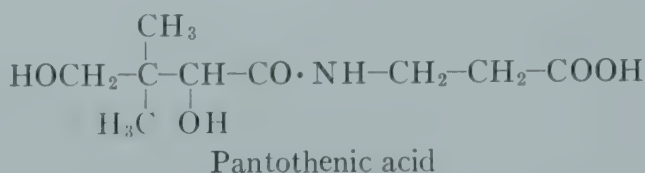


FIG. 2.—*A*, external appearance of rat after being on a pyridoxine-deficient regimen for about 6 weeks. Note normal appearance of fur, in contrast to riboflavin-deficient animal in Fig. 1. At this time the only change, aside from some failure to grow, is found in the extremities, where scaling, *B*, is noted. This appears microscopically, *C*, and hyperkeratosis and acanthosis with epithelial proliferation just above the basal-cell layer. From Follis (60). (Reproduced by permission of Dr. Follis and Charles C Thomas.)

active form of the vitamin, better results might be more consistently obtained.

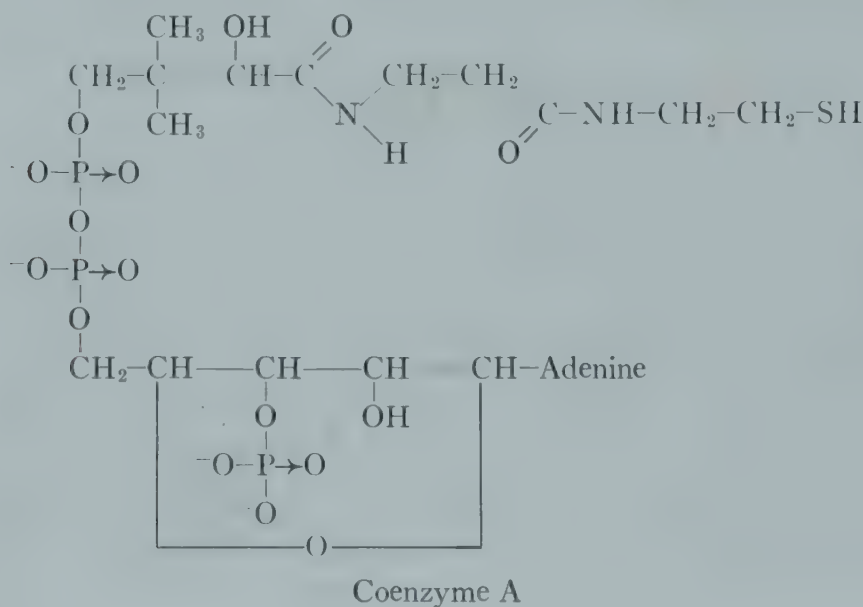
In most animals intestinal flora synthesize vitamin B₆ to help supply normal requirements for the vitamin.

e) *Pantothenic acid*.—Pantothenic acid (filtrate factor), another vitamin B factor, consists of β -alanine united by a peptide linkage to a branched-chain hydroxy fatty acid, pantoic acid, as shown below (246, 244, 245):



It is stable to heat and light and is found in all tissues. The richest known natural source of this vitamin is royal jelly of bees.

Biochemically, it is a major structural component of coenzyme A (139), which is of great importance for acetate utilization in the synthesis of a host of important biochemical compounds such as acetylcholine, steroids, and fats:



Furthermore, pantothenic acid may play a role in the utilization of copper for hair growth and melanin formation. Hundley and Ing (105) find that the skin of pantothenic acid-deficient rats may contain five times

as much copper as normal rat skin, and they suggest that graying and poor hair growth in these deficient rats result from faulty copper utilization in the skin so that copper accumulates. Singer and Davis (201) suggested even earlier that pantothenic acid might be involved in the process of linking copper to the protein moiety of phenol oxidases, because the addition of pantothenic acid to the diet of dried milk fed to copper-deficient rats restored inhibited pigment formation

as well as copper supplementation did.

Sullivan and Nicholls (221) carefully studied the cutaneous effects of pantothenic acid deficiency in the rat. Circumocular hair loss occurs (spectacle alopecia), together with similar alopecia in the preauricular region and on the sides of the snout. Graying also develops symmetrically first in these same general areas, and the fur becomes

dull and coarse (Fig. 3). Generalized scaling and an erythematous dermatitis may also appear. Histologically, hyperkeratosis, acanthosis, crusting and intraepidermal edema in focal distribution, and dilatation of hair

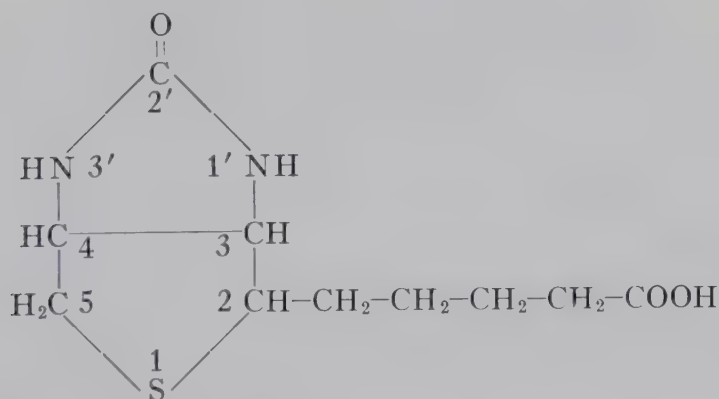
follicles are observed. Later in the deficiency, atrophy of the epidermis can occur.

In the rat (149) and hamster (186) red incrustations, probably of coproporphyrin, accumulate about the nose and snout hairs of deficient animals as a result of chromodacryorrhea from Harderian glands. The skin of the cotton rat has been found more sensitive to low pantothenic acid intake than to any other B-factor deficiency. In these animals death may occur in 6 weeks, with severe dermatitis and cutaneous ulceration resulting from the deficiency (52). In swine, as early as 3 weeks after the institution of pantothenic acid-deficient diets, cutaneous changes have been observed (250). The skin of dogs is apparently more resistant to this deficiency, and no cutaneous changes have been described except for coarsening of hair after 2 months (200).

Pantothenic acid deficiency has not been reported in man, although it has been observed in the monkey (142). Normal requirements of the vitamin can be largely supplied by intestinal-floral synthesis.

Pantoyltaurine is a known competitive inhibitor of pantothenic acid.

f) *Biotin*.—Biotin (vitamin H) is a cyclic acid, containing nitrogen and sulfur, and has the following formula:



Biotin (2'-keto-3,4-imidazolido-2-tetrahydrothiophene-*n*-valeric acid)

Structurally, it possesses both imidazole and thiophene rings. It is a rather stable member of the B complex to heat, alkalis, and acids.

At the beginning of the century it was known that feeding unheated egg-white to animals caused deleterious changes in the

skin and muscular system. Boas in 1927 (16) first postulated a dietary protective factor against this syndrome. György (84) studied in great detail the egg-white injury syndrome and proved Boas' hypothesis to be correct. Du Vigneaud finally determined the structural formula (48) of this anti-egg-white injury factor, and it was soon synthesized (92).

The factor in raw egg-white that inactivates biotin is called "avidin" and has been crystallized (49, 172). It is a glycoprotein that forms a complex with biotin which cannot be absorbed from the intestine, although, on parenteral injection, it can cure the biotin-deficiency syndrome. Curiously, the avidin-biotin complex has considerable lysozyme activity (57).

Lichstein has recently reviewed in critical detail the biochemical functions of biotin, especially in some decarboxylases and deaminases (137). There is considerable evidence that this vitamin, too, functions as a relatively simple organic coenzyme molecule.

Sullivan and Nicholls (220) described in detail the skin changes developing in young rats on a diet containing 30 per cent dried egg-white powder. These changes commenced after 3–5 weeks, with erythema and

roughness of the skin and dulling of the coat. Generalized brown, greasy scaling then appeared, together with symmetrical alopecia, developing first over the chin, neck, and anterior ventral surfaces (Fig. 4, A). Muscle spasticity also was conspicu-

ous, and muscle atrophy and necrosis and increase in sarcolemma nuclei have been seen histologically (217). These muscle changes were analogous to those occurring in vitamin E deficiency.

Microscopically, the skin shows hyperkeratosis, uniform acanthosis, edema of the corium, and follicular dilatation and plugging with sudanophilic keratin material (Fig. 4, *B*). Late in the syndrome the epi-

recently made detailed histologic observations on mouse skin in biotin deficiency and has found changes quite similar to those observed in the rat.

In hamsters (37) biotin deficiency also leads to angular stomatitis. In monkeys (237) with acute biotin deficiency, a scaling dermatitis has been noted, while with chronic deficiency there is only gradual thinning of hair and loss of pigmentation.



FIG. 3.—Head of rat which had been on a pantothenic acid-deficient diet for about 5 weeks. Note symmetric graying (achromotrichia) of hair about eyes, ears, and nose. This usually spreads to involve the entire head; later alopecia occurs. From Follis (60). (Reproduced by permission of Dr. Follis and Charles C Thomas.)

dermis becomes atrophic, the sebaceous glands decrease in size, and small superficial ulcers appear. In mice (165) and rabbits (131) changes similar to those in the rat have been described. In pigmented mice, biotin deficiency leads to achromotrichia as well as alopecia (178), and very early in the syndrome there is a striking failure of the plucking stimulus to initiate a new hair cycle, even though such a cycle can develop spontaneously later. Montagna (158) has

In man, experimental feeding daily of 200 gm. of dehydrated egg-white to four volunteers caused temporary, fine, non-pruritic scaling after 3–4 weeks in all cases (222). A peculiar grayish coloration of the skin, atrophy of lingual papillae, anorexia, insomnia, lassitude, precordial distress, and muscle pain were also noted in these experiments. All these effects were promptly relieved by biotin therapy.

Spontaneous biotin deficiency in man has

not been observed unequivocally. The intestinal flora usually supply ample amounts of the vitamin for normal requirements.

György (85) early pointed out that seborrheic dermatitis in infants, especially the severe form called "Leiner's disease," bears features analogous to biotin-deficiency dermatitis in rats. Thélin (226) has reported

one case of infantile seborrheic dermatitis which responded well to biotin therapy; and Brown (23) has claimed success in the treatment of a few cases of infantile eczema with biotin. In general, however, seborrheic dermatitis has not been benefited by biotin therapy (157, 153).

g) *Inositol*.—Inositol, a cyclic polyalcohol

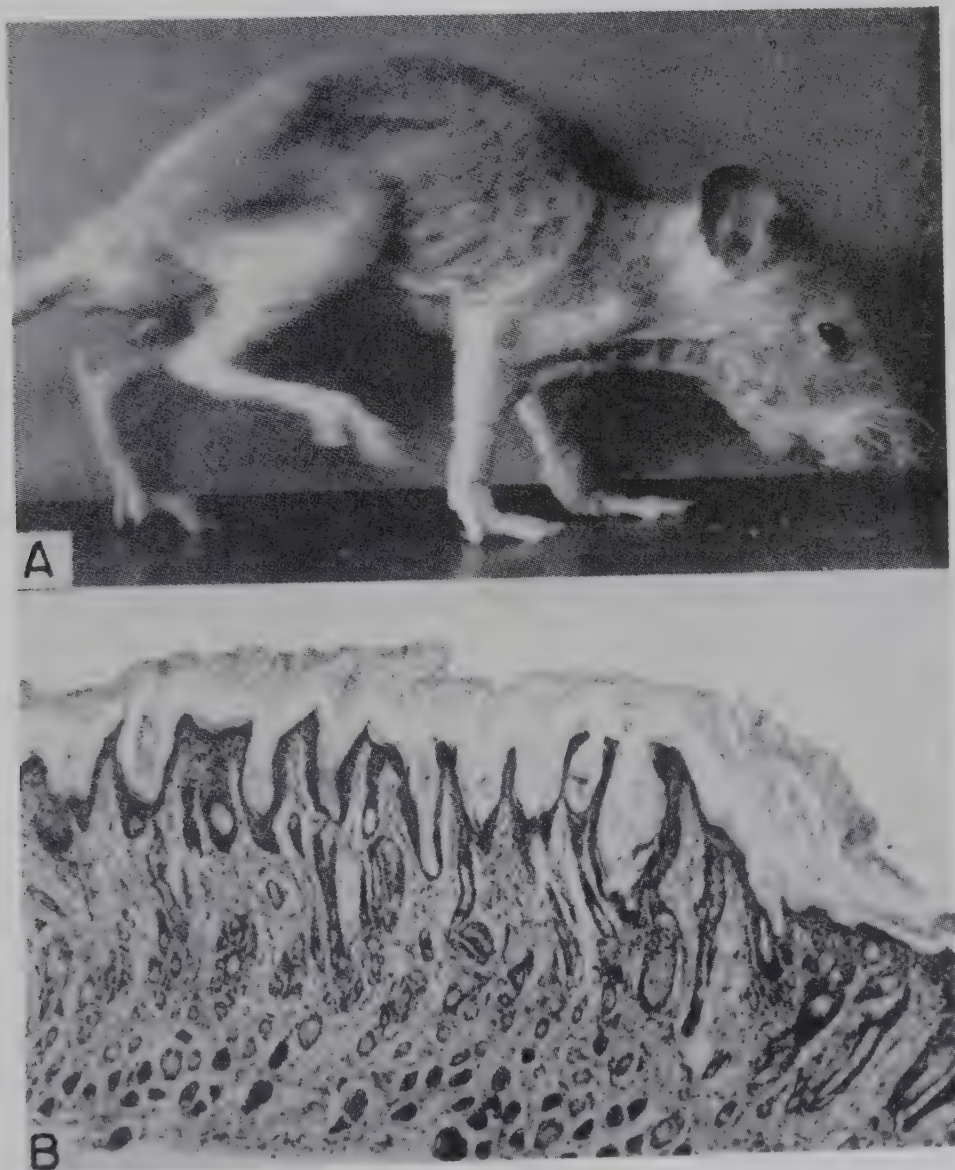
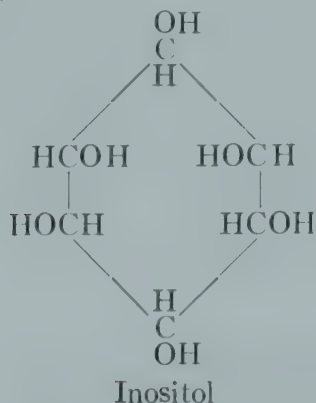


FIG. 4.—Biotin deficiency. *A*, external appearance of a rat which had been placed on a diet containing 30 per cent egg-white. There is complete absence of hair, and the entire body is covered by greasy yellow scales. Note also the humped position and gait. *B*, microscopic picture of skin from late stage similar to that depicted grossly. Note hyperkeratosis, with dilatation of the orifices of the hair follicles. This results in a peculiar finger-like appearance of the epithelium. There is relatively little change in the underlying corium. From Follis (60). (Reproduced by permission of Dr. Follis and Charles C Thomas.)

member of the B complex, has the following structure:



It is widely distributed with phospholipids in animal tissues. It was recognized as an essential nutrient in 1940, when Woolley (257) described alopecia in mice placed on diets deficient in this factor.

Inositol exerts a protective action against fatty infiltrations of the liver in animals (88, 53, 70). In man, the lipotropic properties of inositol have also been noted (1).

Attempts to cure human alopecia with this substance have failed (233), although the inositol content of the hair of balding men has been reported to be low (166).

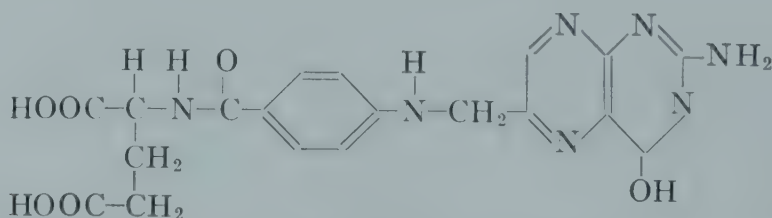
It is of interest to point out the structural similarity between inositol and γ -hexachlorocyclohexane. It has been suggested that this potent antiparasiticide

relationships and it is chiefly a structural component of the phospholipid, lecithin, rather than actively functioning, as do most vitamins, in very tiny amounts. It plays important roles in transmethyations and in the form of acetylcholine.

h) Para-aminobenzoic acid.—Para-aminobenzoic acid, folic acid (*L. casei* factor), and vitamin B₁₂—some other well-known constituents of the B complex—will be considered together because of their close biological interrelationships.

Para-aminobenzoic acid was first suggested as an essential nutrient in 1941 by Ansbacher (7), who produced achromotrichia in rats on a synthetic diet which could be reversed by administration of this substance.

i) Folic acid.—In 1945 Angier *et al.* (5) described the structure and synthesis of the *L. casei* factor, an essential growth factor for bacteria such as *Lactobacillus casei* and *Streptococcus fecalis*, which had earlier been shown to be identical with folic acid, a substance derived from leafy vegetables (156), which was an essential antimacrocytic anemia factor in chicks, monkeys (46), and rats (41). The structure shown in the formula below is pteroylglutamic acid, which has incorporated in it a molecule of para-aminobenzoic acid:



Pteroylglutamic acid; N-[4-{ [(2-amino-4-hydroxy-6-pteridyl) methyl] amino} benzoyl] glutamic acid

functions by competing metabolically with inositol.⁶

Choline, although often considered part of the B complex, is another lipotropic substance. It will not be considered here in detail, because it has no clear-cut cutaneous

Folic acid is an indispensable factor in all tissues, and para-aminobenzoic acid is necessary for its formation. Synthesis by intestinal flora usually supplies the body's normal requirements for folic acid.

Biochemically, folic acid is linked with (1) the biosynthesis of purines and pyrimidines, which are important structural components of nucleic acids; (2) the inter-

6. S. Kirkwood and P. H. Phillips, The anti-inositol effect of γ -hexachlorocyclohexane, *J. Biol. Chem.*, **163**:251-54, 1946.

conversion of glycine and serine; and (3) transmethylation reactions. The biochemical action mechanism of folic acid in these reactions has been dealt with by Jukes (111).

Folic acid antagonists, such as aminopterin, cause striking suppression of blood cell formation, stomatitis, and hemorrhagic manifestations. The latter, of course, is reflected in the skin as purpura. Gubner (81) has suggested that the folic acid antagonists also retard proliferation of epithelial cells, and he has described beneficial effects, presumably on this basis, of aminopterin therapy in psoriasis.

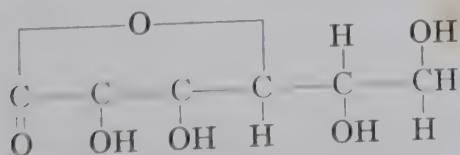
j) *Cyanocobalamin*.—The vitamin B₁₂ group of cobalt-containing pigments has been identified as the extrinsic antipernicious anemia factor (205, 181). Their complex chemical nature and various structural forms and possible pharmacological action are discussed in the following publications: 112, 176, 9, 114, 68, 231, 21, 113, 22. The interrelationships between folic acid and vitamin B₁₂ have been discussed recently by Vilter *et al.* (232).

The relationship of vitamin B₁₂ to the skin and its disorders has not yet been adequately explored, although little can be expected therapeutically, in view of the general lack of striking specific cutaneous effects of liver extract therapy.

k) *Amounts present in skin*.—Lee, Lerner, and Halberg (132) have recently reviewed the quantitative aspects of the B vitamins in normal human skin and give the results of their own measurements of the amounts present. Their figures, in comparison with the results of other investigators, are presented in Table 1.

2. Ascorbic Acid

Ascorbic acid (vitamin C) is a water-soluble, fat-insoluble keturonic acid derivative with the structure illustrated below:



Structural formula of vitamin C (ascorbic acid)

The chemical nature of vitamin C was determined in 1932 by King and Waugh (121), although Szent-Györgyi had earlier found the same material in adrenal glands, cabbage, and citrus fruits (223). Its synthesis was announced soon afterward (179, 97). Ascorbic acid is very easily oxidized to dehydroascorbic acid, especially in alkaline solutions in the light. Copper strongly catalyzes this oxidation, while meta- and pyrophosphates inhibit it.

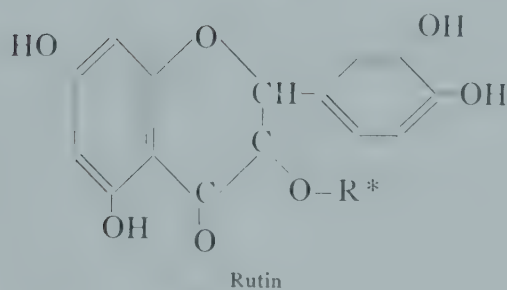
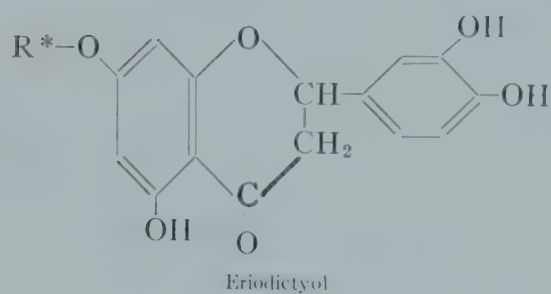
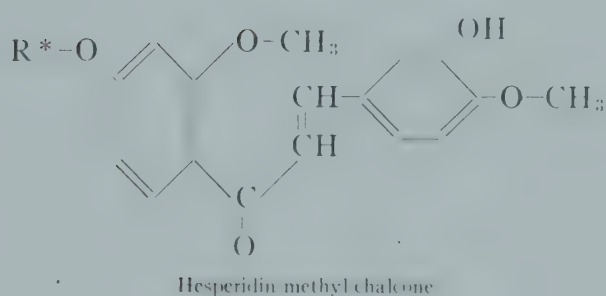
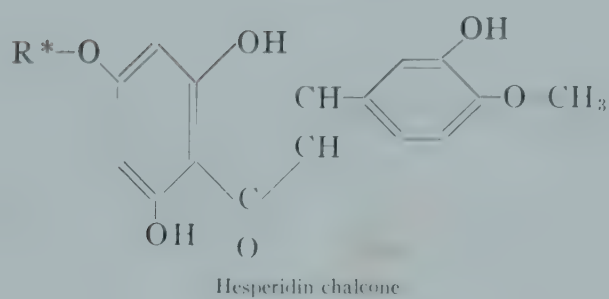
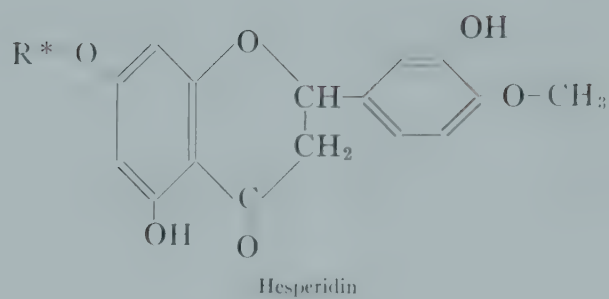
With the exception of monkeys, guinea pigs, and man, animals are able to synthe-

TABLE 1
AMOUNTS OF B VITAMINS IN SKIN (132)

VITAMIN	AMOUNTS IN γ /GM OF DRY SKIN	
	Lee <i>et al.</i>	Literature Data
B ₁₂	0.021
Folic acid.....	0.11	2.3 - 2.7
Riboflavin.....	1.9	2.1 - 3.4
Nicotinic acid.....	15.0	18.0 - 24.0
Calcium pantothenate.....	3.7	6.1 - 9.3
Biotin.....	0.046	0.023- 0.085
Thiamine-HCl.....	0.3	0.95 - 1.7
Pyridoxine.....	0.18 - 0.66
Inositol.....	230 -790

size sufficient ascorbic acid for their biological needs. However, it has been claimed that a vitamin C-deficiency-like syndrome has been produced in mice and rats with glucoascorbic acid (259), a possible metabolic antagonist of ascorbic acid.

Biochemically, ascorbic acid is believed to function as an oxidation-reduction potential regulator in living tissues. It is kept largely in the reduced state in tissues by sulfhydryl compounds, such as glutathione. It can reduce a number of biologically important substances, such as quinones, biliverdin, and oxyhemoglobin. Ascorbic acid also has important functions in the metabolism of certain aromatic amino acids, such as tyrosine and tryptophane, and products derived therefrom, as reviewed by Butt, Leary, and Wilder (27).



* R = a sugar

Structural formulae of compounds with vitamin P activity

The relationship of vitamin C to melanin formation has already been discussed in chapter 22.

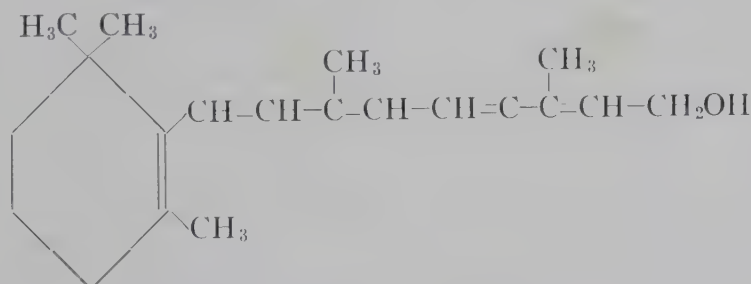
Vitamin C deficiency, as is well known, causes scurvy, one of the earliest recognized specific dietary-deficiency diseases. The manifestations of this disease, including defective wound healing, are based largely on disturbances in the formation and maintenance of normal intercellular substances. The relationships of ascorbic acid to ground substance and collagen formation have already been discussed (pp. 443-44). Only the cutaneous features of this deficiency syndrome will be considered here to any extent. Early in the deficiency, along with lassitude, weakness, and muscle and joint pains, hyperkeratotic follicular papules on the buttocks and calves have been described which closely resemble similar cutaneous lesions often ascribed to vitamin A deficiency. To explain this similarity, Frazier and Marmelszadt have suggested that vitamin C may play a role in the utilization of vitamin A (66). Later in the disease hemorrhagic phenomena develop in the skin and internal organs. The gums become swollen, red, and bleed easily. General skin pallor

citrus fruit rinds and isolated the active principle, "citrin," and identified two active chemical components, hesperidin and eriodictyol. Since then other related compounds whose structures are shown on the facing page have been shown to have vitamin P activity.

Vitamin P deficiency has been produced in man (189, 190), and skin lesions characterized by perifollicular petechiae and purpura over pressure areas have been described. Some claims for the value of vitamin P in the treatment of allergic, infectious, and other purpuras have been made (128, 107, 154, 76, 138).

4. Vitamin A

In 1913, McCollum and Davis (145) and Osborne and Mendel (169) noted the presence of a growth factor for rats in certain fats. Six years later, Steenbock (211) suggested that this factor, called vitamin A, was carotene, a yellow plant pigment. In 1930 carotene was shown to be provitamin A (161); and Karrer (117, 118) elucidated the chemical structure of both carotene and vitamin A.



Structural formula of vitamin A₁

related to accompanying anemia is also characteristic of the syndrome.

3. Vitamin P

Vitamin P is a designation applied to a group of water-soluble flavone glycosides from various plant sources which are believed necessary to maintain normal capillary permeability and fragility. Szent-Györgyi and his associates (187) first discovered this vitamin factor in paprika and

Two active forms of vitamin A occur in tissues—vitamin A₁, whose structure is shown here, and vitamin A₂.

Vitamin A₂ is a dehydrogenation product of A₁ and occurs mainly in fresh-water fishes. Both these substances are colorless, though they fluoresce under ultraviolet light. The plant provitamins are the following group of hydrocarbon pigments: α -, β -, and γ -carotenes, cryptoxanthine, and echinone.

In addition, liver oils contain kitols (inactive divitamins A), which can be converted to active forms by heating (57). The plant provitamins are broken down and oxidized to vitamin A in the liver by an enzyme, carotenase (167), which splits the prohydrocarbons into two halves. This conversion also occurs in the intestinal wall. β -Carotene is the most potent of these plant precursors, because it can theoretically yield 2 moles of vitamin A, whereas the others can yield only 1. However, the actual conversion of β -carotene to vitamin A is not 100 per cent efficient, and the mechanism of this conversion is not entirely known. In severe liver disease, diabetes, and hypothyroidism the conversion of provitamins A into the active form may be impaired.

The vitamins and provitamins A are fairly stable to heat but are susceptible to destruction by photo-oxidation.

The vitamin A esters of foods are hydrolyzed by esterase in the small bowel and during absorption are re-esterified with fatty acids. The plant provitamins are absorbed less readily and completely and require bile salts for the process. Unsaturated fatty acids hasten their absorption, whereas mineral oil interferes with the process. The tocopherols have a sparing action on vitamin A by inhibiting its oxidative destruction in the gastrointestinal tract. Ninety-five per cent of the storage of vitamin A in mammals is in the liver, while the provitamins are stored in both liver and adipose tissue.

The functions of vitamin A in biological systems are not too well known except for its role in visual processes (238). Recently a theory has been proposed which claims that vitamin A exerts a restraining effect on the dehydrogenation of cholesterol and sterols to β -naphthalenic compounds. A substance with an absorption spectrum like that of β -naphthalenic compounds has been detected in the livers of vitamin A-deficient rats.⁷ This theory is of interest, in view of the well-known acnegenic nature of some

naphthalene derivatives and the production of hyperkeratosis or X disease in cattle by dietary intake of penta-, hexa-, or octachloronaphthalene.⁸

The important role of vitamin A in preventing keratinizing metaplasia of epithelial tissues has already been considered on pages 382-85. Observations and interpretations of changes in vitamin A deficiency in experimental animals were reported in great detail in a series of papers by Wolbach and co-workers (251-56).

In summary, vitamin A was found essential for (1) maintenance of normal epithelium in the eye, in the upper respiratory, gastrointestinal, and genitourinary tracts, and in the ducts and acinar tissues of secretory glands; (2) promotion of normal bone and tooth-enamel development; and (3) maintenance of the dark-adaptation visual cycle.

Despite changes in epithelium at other sites in vitamin A-deficient animals (namely, atrophy followed by keratinizing metaplasia), cutaneous alterations, in contrast to what has been reported in man, have not been conspicuous. Sullivan and Evans (216) commented that, since skin is already keratinized, such changes are not to be expected. They then showed that extensive hyperkeratinization develops in rats if, before vitamin A deficiency, the skin is rendered atrophic by heat-stable vitamin B-complex deficiency.

In man the characteristic, generally accepted features of vitamin A deficiency have been xerophthalmia, keratomalacia, nyctalopia, and follicular hyperkeratotic papular dermatitis (phrynoderma). Frazier and co-workers in a series of classic papers have reported in considerable detail their observations and opinions about human vitamin A deficiency, especially as it affects the skin in the Orient (62-67). Urbach (230) reviews in detail the cutaneous manifestations

7. J. S. Lowe, R. A. Morton, and R. G. Harrison, Aspects of vitamin A deficiency in rats, *Nature*, 172:716-19, 1953.

8. D. Sikes, J. C. Wise, and M. E. Bridges, The experimental production of "X disease" (hyperkeratosis) in cattle with chlorinated naphthalenes and petroleum products, *J. Am. Vet. M. A.*, 121: 337-44, 1952.

of vitamin A deficiency in man that have been reported.) The difficulty in demonstrating the presence of vitamin A in the skin is discussed on page 330.)

Some doubt as to the specificity of the cutaneous changes reported in man for uncomplicated vitamin A deficiency alone has been engendered by the failure to produce features of the deficient state by vitamin A-deficient experimental diets in volunteers (20), as well as by the conspicuous relative absence of cutaneous changes in vitamin A deficiency in animals.

Sjögren's syndrome (203) has been considered to be a manifestation of vitamin A deficiency (210). Frazier and Marmelszadt (66) have suggested that almost all skin disorders featuring follicular hyperkeratotic papules may, in the last analysis, prove to be related to faulty vitamin A metabolism of one sort or another. Others, however, claim that follicular hyperkeratosis is characteristically seen on exposure to cold or in general caloric undernutrition and that vitamin A or C deficiencies merely sensitize the skin to these effects of cold or semistarvation.⁹ Still others have recently reviewed the evidence against vitamin A as the cause of phrynoderma and find that, in rats, phrynoderma-like changes are more consistently produced by essential fatty-acid-deficient diets than by vitamin B₆ or vitamin A deficiency.¹⁰

The claim by Kaunitz and Slanetz that a vitamin A-free lard distillate could prevent vitamin-deficiency keratomalacia in rats¹¹ has recently been retracted by these authors, working with Herb and Riemen-schneider, after they demonstrated, on more

careful analysis, the presence of "typical vitamin A" in the lard distillates used.¹²

(Hypervitaminosis A may cause disturbances in growth of keratinous structures (194), in addition to systemic disturbances (230).)

5. Vitamin D

Windaus *et al.* (247) demonstrated that ultraviolet light irradiation of 5-6, 7-8 unsaturated sterols or provitamins produces fat-soluble substances with vitamin D potency after Steenbock and Black (212) and Hess and Weinstock (96) had shown that ultraviolet irradiation of food materials imparts vitamin D activity to them. Of these D vitamins, calciferol (vitamin D₂, viosterol), produced by irradiation of ergosterol, a vegetable sterol, and vitamin D₃ (found in fish-liver oils), formed by irradiation of dehydrocholesterol, are biologically the most important and are widely used for the cure and prevention of rickets, the specific vitamin D-deficiency syndrome. Their structures are shown on the following page.

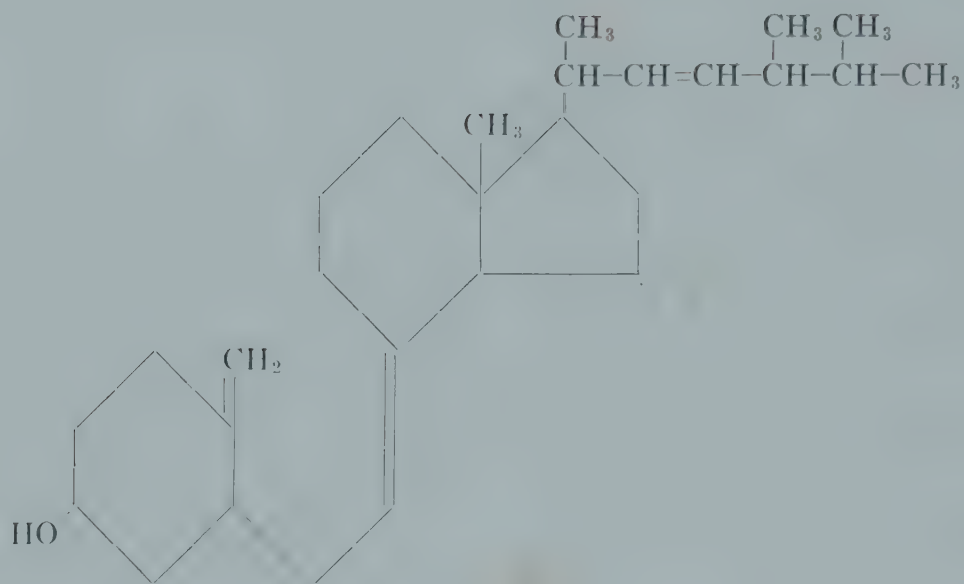
Unlike fat-soluble vitamin A, the D vitamin preparations are relatively stable to oxidation. Biologically, vitamin D plays an important role in calcium and phosphorus metabolism. There is evidence that this is at least partly based on an enhancing action upon calcium absorption from the intestine (164, 77). Although no specific cutaneous effects have been ascribed to vitamin D deficiency, there are some important relationships of this vitamin to the skin. First, the antirachitic effect of sunlight irradiation is based upon conversion of vitamin D precursors in the surface lipid film to active forms, which are then either ingested or absorbed percutaneously. Second, vitamin D₂ therapy has been variably claimed to be beneficial in a number of skin disorders, including psoriasis (30, 24, 125, 260, 50), scleroderma (39, 173), pemphigus (225, 135), and sarcoidosis (40, 182, 163), and has definitely proved to be of value in lupus vul-

9. R. A. McCance and A. M. Barrett, The effect of undernutrition on the skin, *in*: Studies in undernutrition, Wuppertal 1946-9, pp. 83-96 (Special Report Ser. Medical Research Council, No. 275 [1951]).

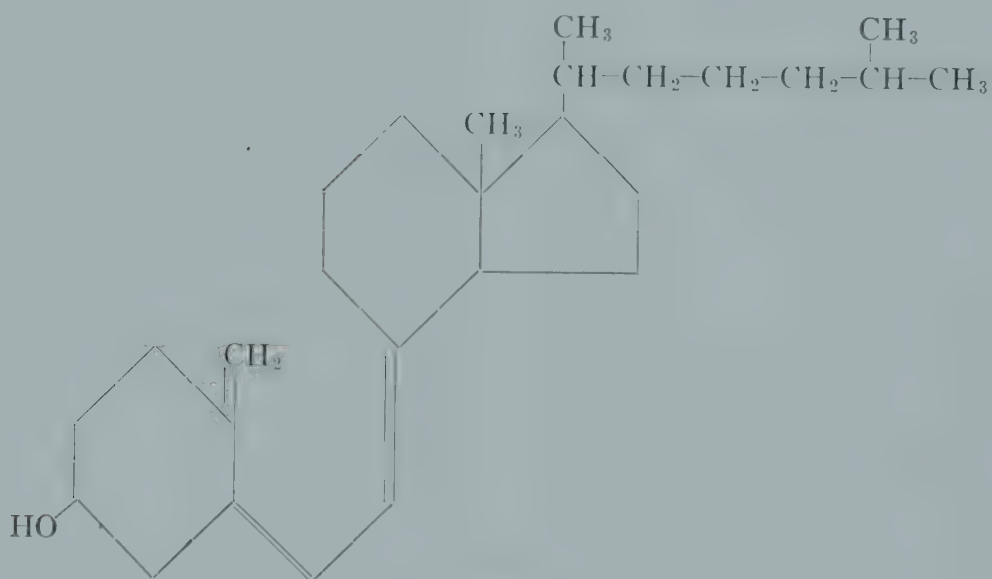
10. V. Ramalingaswami and H. M. Sinclair, The relation of deficiencies of vitamin A and of essential fatty acids to follicular hyperkeratosis in the rat, *Brit. J. Dermat.*, **65**:1-21, 1953.

11. H. Kaunitz and C. A. Slanetz, An unknown factor with vitamin A activity distilled from lard, *J. Nutrition*, **42**:375-89, 1950.

12. S. F. Herb, R. W. Riemen-schneider, H. Kaunitz, and C. A. Slanetz, Nature of the "vitamin A-like factor" in lard, *J. Nutrition*, **51**:393-402, 1953.



Vitamin D₂ (calciferol)



Vitamin D₃

Structural formulae of the principal vitamins D

garis (47, 32). In this latter disease, the beneficial effect solely against cutaneous forms of tuberculosis is not a direct one against the bacilli but rather an indirect action probably based on specific electrolyte changes induced in the skin. This latter concept recalls the old Gerson low-salt dietary regimen for this disease (p. 498).

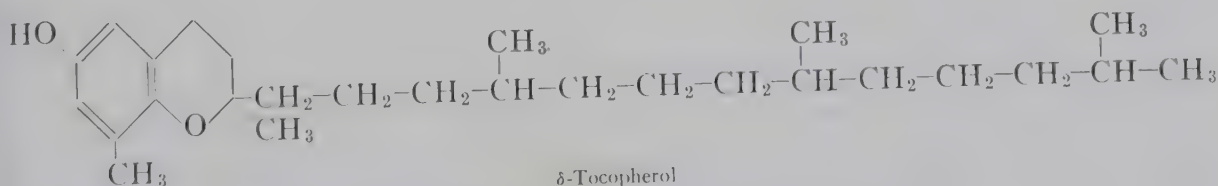
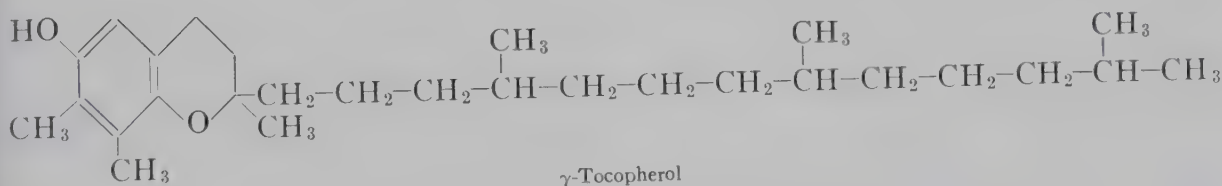
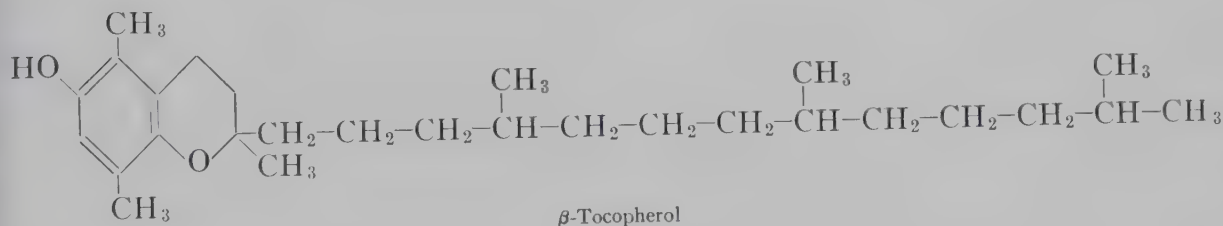
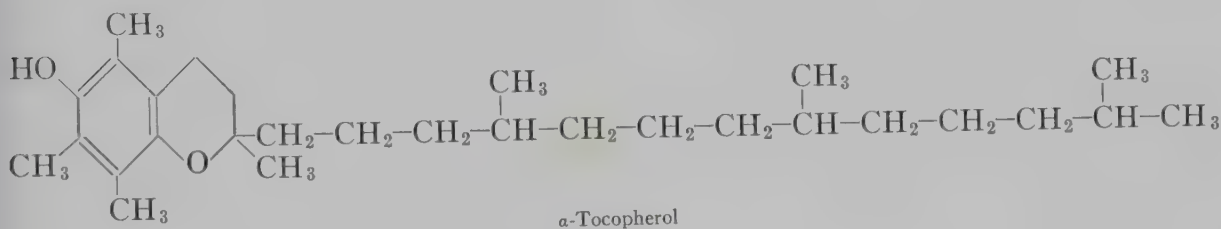
6. Vitamin E

The fat-soluble, heat-stable vitamins of the E series are chroman derivatives, as illustrated by the structure of α -tocopherol, the most active member of the series, shown here:

These substances act as potent antioxidants in fatty material. Their biochemical activity otherwise is not well understood. Plant oils are rich sources of tocopherols, wheat-germ oil being a particularly potent source.

No specific cutaneous functions are known. Human vitamin E deficiency has not been described.

Tocopherol therapy has been proposed for a vast array of diseases, including skin disorders of many kinds; but initial expectations for their value, especially in lupus erythematoses, necrobiosis lipoidica diabetorum, and phlebotic diseases (199, 25), have not survived well the test of time.



Structural formulae of the tocopherols (vitamin E)

In animals they have important roles in the reproductive and muscular systems (60).

The chemistry and synthesis of vitamin E were elucidated through the work of Evans, *et al.* (56) and Karrer *et al.* (116).

7. Vitamin K

Vitamins of the K series are fat-soluble, heat-stable, yellow derivatives of 1,4-naphthoquinone, which are easily oxidized in the presence of light. A synthetic member of the

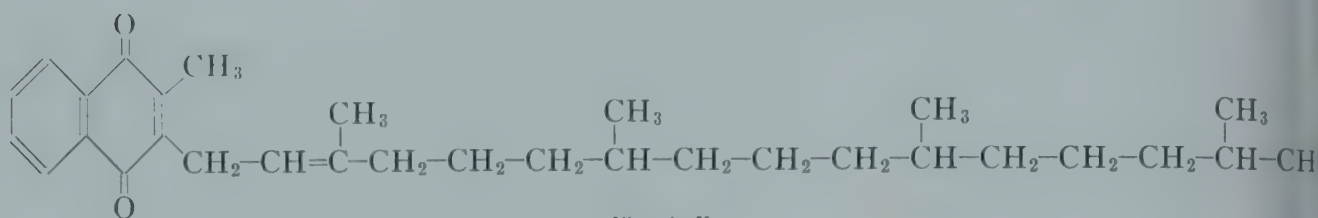
series, 2-methyl-1,4-naphthoquinone (menadione), is the most potent (8).

The only known specific function of this vitamin is the maintenance of the hepatic prothrombin synthesis so necessary for normal blood clotting (206, 4, 123, 160, 42).

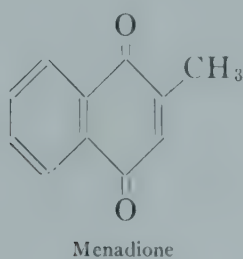
derivatives and related compounds, such as dicumarol (170), also deserves mention here.

C. ESSENTIAL FATTY ACIDS

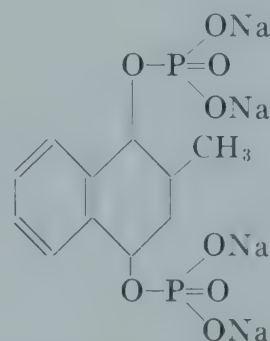
Knowledge of the nutritional role of unsaturated fatty acids commenced in 1929



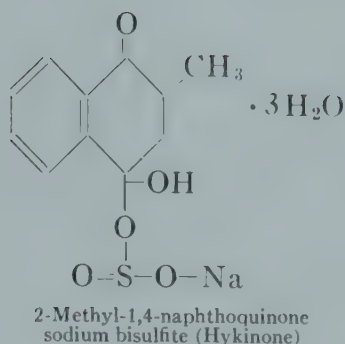
Vitamin K₁



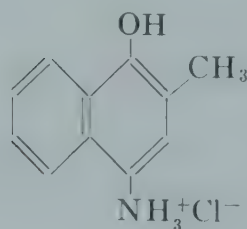
Menadione



Tetrasodium 2-methyl-1,4-naphthohydroquinone diphosphoric acid ester ("Kappadione," Synkayvite)



2-Methyl-1,4-naphthoquinone sodium bisulfite (Hykinone)



2-Methyl-4-amino-1-naphthol hydrochloride (Synkamin)

Structural formulae of some substances having vitamin K activity

Vitamin K deficiency in animals and man is characterized by hemorrhagic phenomena which may cause extensive purpura in the skin (115). Bile salts are necessary for the intestinal absorption of vitamin K, as well as for the absorption of the other fat-soluble vitamins.

The well-known antagonism of vitamin K to the anticoagulant action of coumarin

with the description by Burr and Burr (26) of a disease syndrome in rats produced by a fat-free diet. This syndrome consisted of growth failure after 4–5 months and renal and skin lesions. Ten drops of lard daily cured all manifestations of this deficiency. Linoleic acid was then specifically found curative (26), and later linolenic and arachidonic acids were found able to substitute

for linoleic acid in growth promotion. The structures of these fatty acids are illustrated in the formulae below:



Linoleic acid



Linolenic acid



Arachidonic acid

Biochemically, the functions of the essential fatty acids are not clear, but Schœnheimer (192) has shown that, in contrast to other fatty acids, these essential ones cannot be synthesized within the body.¹³

Rats on essential fatty-acid-deficient diets consume unusually large amounts of water (26). After several months they develop scaling dermatitis, especially on the feet, ears, and tail, and sometimes alopecia also. The skin changes have been claimed to be identical with those of rat acrodynia resulting from pyridoxine deficiency (78, 13, 177). The relationship of these factors to one another is reviewed by Gross (78).

Mice show skin changes from essential fatty-acid deficiency similar to those seen in rats (242). Young dogs on such deficient diets (90) also develop flaking desquamation of the skin and show coarse, dry hair, in addition. Croton oil also more readily irritates the skin of such deficient dogs, and the iodine number of their serum fat is low, whereas the skin fat shows no change from normal in iodine number.

In man there have been claims by Hansen (89), Cornbleet (38), and Finnerud *et al.* (58), among others, of beneficial effects of essential fatty acids in some cases of dermatitis, especially infantile eczema and atopic dermatitis. However, Taub and Zakon (224), Epstein and Glick (54), and Ginsberg, Bernstein, and Iob (72), among others, failed to note improvement with such therapy.

feeding was found to cause a rise in these low iodine-number values.

The popularity of internal lard therapy of dermatitis at the present time appears to be waning. In the writer's own experience neither topical nor internal lard therapy had any specific beneficial effect on atopic dermatitis.

D. MINERALS AND TRACE ELEMENTS

At least eighteen elements of the periodic table are known to be essential dietary factors for animals (60). In the following discussion of the cutaneous relationships of these essential elements, the basic structural elements of all tissues—carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur—and the three elements most fundamental among body fluid electrolytes—sodium, potassium, and chlorine—will not be further commented upon here. Of the remaining nine essential elements, dietary deficiencies of four—calcium, magnesium, zinc, and copper—have obvious direct cutaneous repercussions, whereas specific deficiencies of the other five—fluorine, iodine, manganese, iron, and cobalt—have not been reported to have such direct primary cutaneous effects and so will not be discussed. Other elements, of course, besides these essential ones occur in the skin, including aluminum, silicon, nickel, arsenic, rubidium, lead, silver, boron, titanium, and strontium. Goldblum, Derby, and Lerner (74) have recently estimated the relative and absolute

13. For possible synthesis in the skin see p. 318.

amounts of metals in skin, hair, and nails. Their results are summarized in Tables 2 and 3.

1. Calcium

Calcium, besides its major roles in the skeletal system and in the physiology of blood coagulation and muscle and nerve irritability, appears to have an important function in controlling capillary permeability via the "intercellular cement substance," which is probably a calcium proteinate (31,

cussion of the role of calcium in relationship to the skin is given on page 503.

2. Magnesium

Magnesium deficiency was first studied by McCollum and co-workers in the rat (147). This element is present in all tissues largely as an intracellular component, being second only to potassium in mineral concentration. It has effects on nerve and muscle irritability opposite to those of cal-

TABLE 2
METALS ASSOCIATED WITH ENZYMES (PER CENT OF DRY TISSUE) (74)

Metal	Skin	Nails	Hair
Zn.....	2.0×10^{-3} to 6.78×10^{-2}	1.16×10^{-2} to 3.08×10^{-1}	6.4×10^{-3} to 5.62×10^{-2}
Mg.....	1.0×10^{-3} to 1.5×10^{-2}	2.3×10^{-3} to 1.10×10^{-2}	1.0×10^{-3} to 1.01×10^{-2}
Cu.....	7.43×10^{-4} to 8.8×10^{-3}	9.4×10^{-4} to 8.1×10^{-3}	3.12×10^{-3} to 1.28×10^{-2}
Fe.....	9.0×10^{-4} to 5.9×10^{-3}	1.8×10^{-3} to 6.5×10^{-3}	8.4×10^{-6} to 1.1×10^{-3}
Mn.....	2.4×10^{-9} to 6.1×10^{-7}	6.3×10^{-8} to 3.3×10^{-7}	1.0×10^{-9} to 5.5×10^{-12}

TABLE 3
INERT METALS (PER CENT OF DRY TISSUE) (74)

Metal	Skin	Nails	Hair
Si.....	0.64 to 2.14	1.7×10^{-1} to 5.4×10^{-1}	3×10^{-2} to 3.6×10^{-1}
Pb.....	2.52×10^{-2} to 1.34×10^{-1}	9.7×10^{-3} to 2.4×10^{-2}	4.1×10^{-3} to 1.0×10^{-2}
B.....	1.7×10^{-3} to 1.72×10^{-2}	7.0×10^{-4} to 6.0×10^{-3}	2.0×10^{-4} to 8.0×10^{-4}
Ti.....	1.06×10^{-4} to 2.77×10^{-4}	1.63×10^{-5} to 3.9×10^{-5}	3.2×10^{-6} to 6.4×10^{-6}
Sr.....	3.2×10^{-9} to 4.5×10^{-6}	1.6×10^{-7} to 3.3×10^{-6}	2.2×10^{-8} to 9.1×10^{-6}
Ag.....	1.7×10^{-8} to 4.5×10^{-8}	4.8×10^{-8} to 6.5×10^{-7}	4.8×10^{-7} to 4.5×10^{-6}
Al.....	5.3×10^{-11} to 4.5×10^{-9}	4.5×10^{-11} to 5.3×10^{-8}	5.5×10^{-12} to 2.1×10^{-9}

262) (p. 64). In experimental dietary calcium deficiency in rats (17, 18, 19) and dogs (151) widespread hemorrhages are a conspicuous feature, probably as a consequence of weakened capillary walls.

Interest in the cutaneous role of calcium was early stimulated by Luithlen's (141) observation that excess calcium administered to animals decreased the skin's reactivity to inflammatory stimuli. Klauder and Brown (122) later partially confirmed this inverse relationship between skin calcium content and irritability, although no such relationship of skin irritability to blood calcium levels could be shown. Further dis-

cussion of the role of calcium in relationship to the skin is given on page 503.

cium. Biochemically, magnesium functions catalytically for a number of enzymes, including phosphatases, phosphorylases, leucylpeptidase, enolase, glucophosphomutase, and carboxylase. The outstanding features of magnesium deficiency in the rat (127) and dog (168) include changes in the skin, kidneys, liver, vascular system, and teeth and hyperirritability of the neuromuscular system.

Sullivan and Evans (214) reported in detail the cutaneous lesions in rat magnesium deficiency. After 1 week of dietary magnesium depletion, erythema and edema developed on the ears, paws, and trunk. Later,

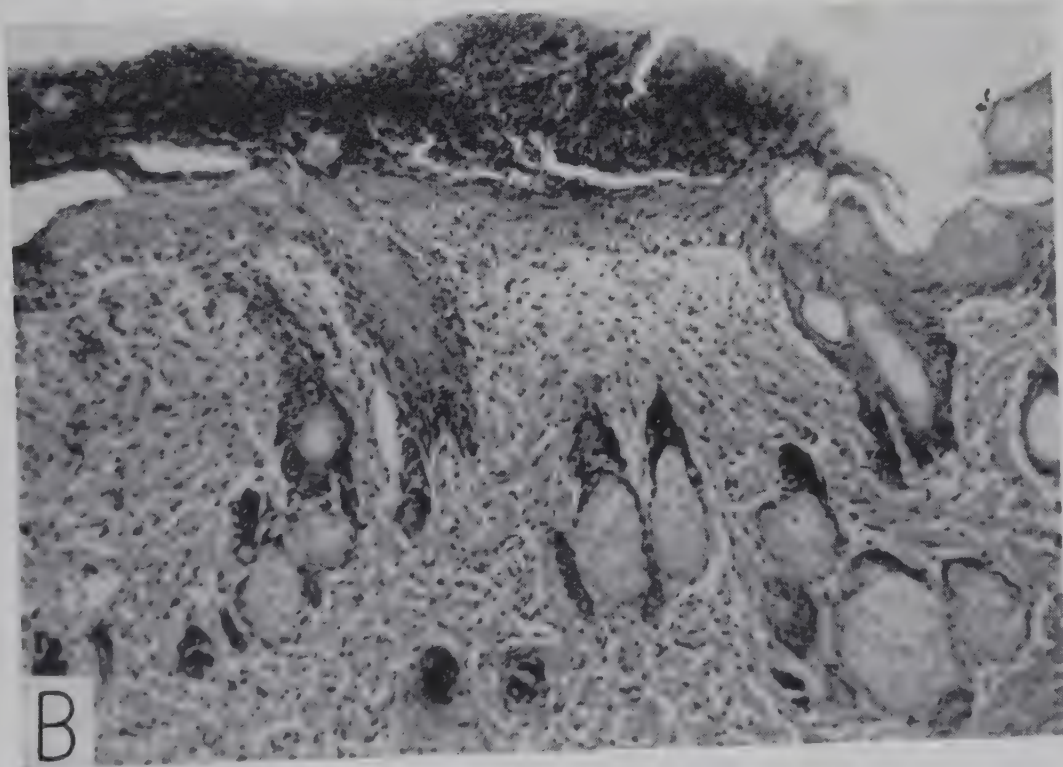
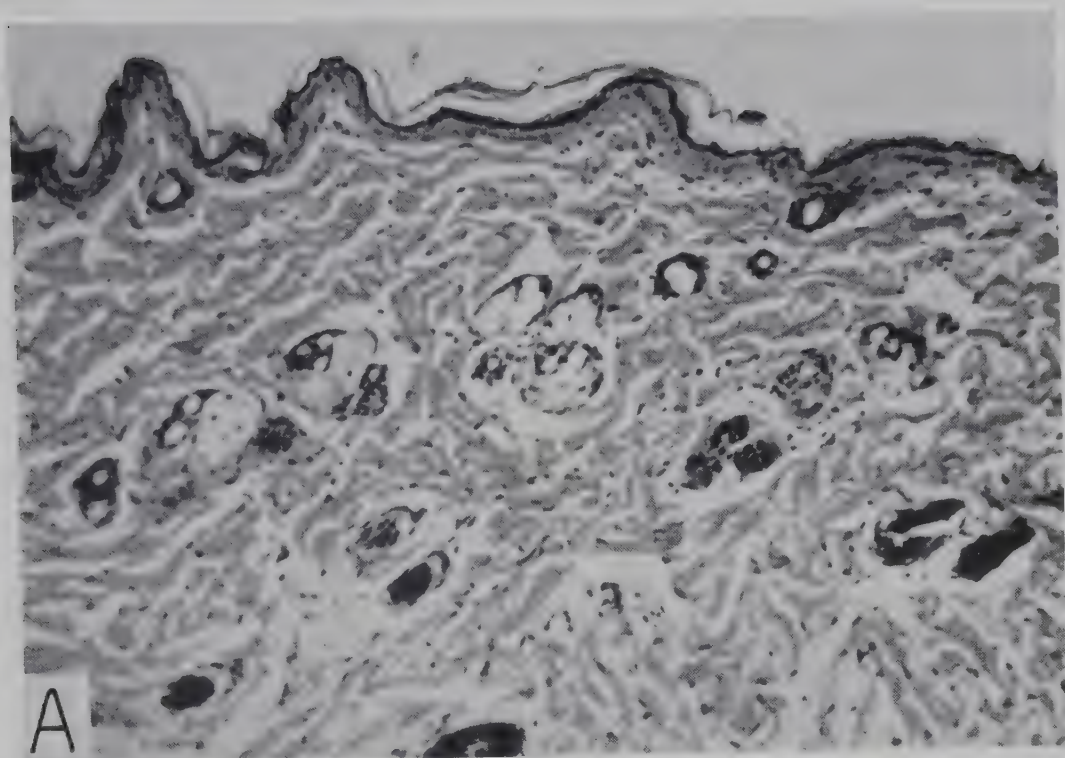


FIG. 5.—*A*, normal skin; *B*, skin in zinc deficiency. There is a crust of partially keratinized cells overlying a layer of thickened epithelium, which shows acanthosis. There is atrophy of the hair follicles, with persistence of the sebaceous glands; in fact, these structures are hypertrophied in comparison with the normal. This animal had been on a zinc-deficient diet for 74 days. From Follis (60). (Reproduced by permission of the author and Charles C Thomas.)

loosely laminated hyperkeratosis and patchy acanthosis occurred, with individual epidermal cells becoming vacuolated and showing pyknotic nuclei. No changes were observed in the sweat and sebaceous glands. Curiously, despite these changes, the magnesium content of the skin was not decreased below normal levels, although serum magnesium levels were reduced (215). These workers were unable to confirm the claim (229) that vitamin B influences the signs of magnesium deficiency. MacCardle *et al.* (144, 143) reported that the magnesium content of the skin is low in chronic disseminated neurodermatitis (p. 510).

3. Zinc

Zinc functions as a cofactor for several enzymes, including carbonic anhydrase (119), uricase (98), and phosphatases (35, 103). Cell nuclei have higher zinc concentrations than the cytoplasm does (57). The liver and pancreas appear to be particularly concerned with zinc metabolism on the basis of studies with radioactive zinc (197, 198, 159).

Todd, Elvehjem, and Hart in 1931 (228) first demonstrated that zinc is essential in the diet of rats, and many further studies on the basis of this work have followed (45, 104, 234, 101, 102, 44). Rats kept on a diet containing only 1.6 parts zinc per million developed growth disturbance, skin changes, and alopecia, all of which could be reversed by the daily addition of 100 γ of zinc to the diet. On the deficient diet, weight gain ceased after 2-3 weeks, and alopecia, together with a scaly dermatosis, commenced about 1 week later. Histologically (61), acanthosis and heavy parakeratotic scales are found in the skin. Vacuolization of some

epidermal cells with pyknosis of their nuclei also occurs. There is a severe atrophy of the hair follicles, while the sebaceous glands, by way of contrast, show a striking hyperplasia (Fig. 5). Changes on the posterior tongue and palate are somewhat reminiscent of those of early leukoplakia. The zinc content of the skin in beriberi has been claimed to be low (57).

Zinc deficiency in man probably never occurs, because of the widespread distribution of zinc in food.

Gross (80) studied the toxic effects of zinc in the rat and noted that chronic excess dietary zinc intake led to the development of skin changes identical with those described in pantothenic acid deficiency and found that pantothenic acid cured this zinc toxicity syndrome.

4. Copper

Copper was proved to be an essential nutrient in 1928 by Hart, Steenbock, Waddell, and Elvehjem (93). It functions in some unknown way as an essential factor for hematopoiesis. Biochemically it is an important cofactor for several enzymes (150). Copper deficiency causes primarily severe anemia and growth retardation. Achromotrichia, hair disturbances, and widespread degeneration of myelin (150) have also been noted. The latter has been observed only in sheep.

The relationship of copper deficiency to deficient melanin formation and keratinization, as well as the role of excess molybdenum intake in producing copper deficiency, has already been discussed on pages 530 and 371. The possible relationships between pantothenic acid and copper have also been considered elsewhere (pp. 541 and 672).

BIBLIOGRAPHY

1. ABELS, J. C.; KUPEL, C. W.; PACK, G. T.; and RHOADS, C. P. Metabolic studies in patients with cancer of the gastro-intestinal tract. XV. Lipotropic properties of inositol, *Proc. Soc. Exper. Biol. & Med.*, **54**:157-58, 1943.
2. ALBANESE, A. A., and BUSCHKE, W. On cataract and certain other manifestations of tryptophane deficiency in rats, *Science*, **95**:584-86, 1942.
3. ANDREWS, J. C., and RANDALL, A. Sulfur metabolism in cystinuria, *J. Clin. Investigation*, **14**:517-24, 1935.
4. ANDRUS, W. DE W.; LORD, J. W., JR.; and

- MOORE, R. A. The effect of hepatectomy on the plasma prothrombin and the utilization of vitamin K, *Surgery*, **6**:899-900, 1939.
5. ANGIER, R. B., *et al.* The structure and synthesis of the liver *L. casei* factor, *Science*, **103**:667-69, 1946.
 6. ANONYMOUS. Vitamin B₆ and seborrheic dermatitis in man, *Nutrition Rev.*, **10**:323-25, 1952.
 7. ANSBACHER, S. *p*-Aminobenzoic acid, a vitamin, *Science*, **93**:164-65, 1941.
 8. ANSBACHER, S., and FERNHOLZ, E. Simple compounds with vitamin K activity, *J. Am. Chem. Soc.*, **61**:1924-25, 1939.
 9. ANSLOW, W. K.; BALL, S.; EMERY, W. B.; FANTES, K. H.; SMITH, E. L.; and WALKER, A. D. The nomenclature of the vitamin B₁₂, *Chem. & Indust.*, p. 574, 1950.
 10. ANTROPOL, W., and UNNA, K. Pathologic aspect of nutritional deficiencies in rats; lesions produced by diets free of vitamin B₆ (pyridoxine) and their response to vitamin B₆, *Arch. Path.*, **33**:241-58, 1942.
 11. BARRON, E. S. G.; MEYER, J.; and MILLER, Z. B. The metabolism of skin. Effect of vesicant agents, *J. Invest. Dermat.*, **11**:97-118, 1948.
 12. BEST, C. H., and TAYLOR, N. B. The physiological basis of medical practice. Baltimore: Williams & Wilkins Co., 1943.
 13. BIRCH, T. W. The relation between vitamin B₆ and the unsaturated fatty acid factor, *J. Biol. Chem.*, **124**:775-93, 1938.
 14. BIRCH, T. W.; GYÖRGY, P.; and HARRIS, L. J. The vitamin B₂ complex: differentiation of the anti-blacktongue and the "P-P" factors from lactoflavin and B₈ (so-called "rat pellagra" factor). I-VI, *Biochem. J.*, **29**:2830-50, 1935.
 15. BISCHOFF, F.; INGRAHAM, L. P.; and RUPP, J. J. Influence of vitamin B₆ and pantothenic acid on growth of sarcoma 180, *Arch. Path.*, **35**:713-16, 1943.
 16. BOAS, M. A. The effect of desiccation upon the nutritive properties of egg white, *Biochem. J.*, **21**:712-24, 1927.
 17. BOELTER, M. D. D., and GREENBERG, D. M. Severe calcium deficiency in growing rats. I. Symptoms and pathology, *J. Nutrition*, **21**:61-74, 1941.
 18. ———. Severe calcium deficiency in rats. II. Changes in chemical composition, *ibid.*, pp. 75-84.
 19. ———. Effect of severe calcium deficiency on pregnancy and lactation in the rat, *ibid.*, **26**:105-21, 1943.
 20. BRENNER, S., and ROBERTS, L. J. Effects of vitamin A depletion in young adults, *Arch. Int. Med.*, **71**:747-82, 1943.
 21. BRINK, N. G.; KUEHL, F. A., JR.; and FOLKERS, K. Vitamin B₁₂: the identification of vitamin B₁₂ as a cyano-cobalt coordination complex, *Science*, **112**:354, 1950.
 22. BROCKMAN, J. A., JR.; PIERCE, J. B.; STOKSTAD, E. L. R.; BROQUIST, H. P.; and JUKES, T. H. Some characteristics of a crystalline compound derived from vitamin B₁₂, *J. Am. Chem. Soc.*, **72**:1042, 1950.
 23. BROWN, A. Effect of egg white and crystalline biotin methyl ester on skin lesions in three infants, *Glasgow M. J.*, **29**:309-16, 1948.
 24. BRUNSTING, L. A. Treatment of psoriasis by ingestion of massive doses of vitamin D, *Proc. Staff Meet. Mayo Clin.*, **13**:280-83, 1938.
 25. BURGESS, J. F., and PRITCHARD, J. E. Tocopherols (vitamin E). Treatment of lupus erythematosus; preliminary report, *Arch. Dermat. & Syph.*, **57**:953-64, 1948.
 26. BURR, G. O., and BURR, M. M. On the nature and role of the fatty acids essential in nutrition, *J. Biol. Chem.*, **86**:587-621, 1930.
 27. BUTT, H. R.; LEARY, W. V.; and WILDER, R. M. Diseases of nutrition; review of certain recent contributions, *Arch. Int. Med.*, **69**:277-343, 1942.
 28. CANNON, P. R.; CHASE, W. E.; and WISSLER, R. W. The relationship of the protein-reserves to antibody production. I. The effects of a low-protein diet and of plasmapheresis upon the formation of agglutinins, *J. Immunol.*, **47**:133-47, 1943.
 29. CARTWRIGHT, G. E.; WINTROBE, M. M.; JONES, P. J.; LAURITSEN, M.; and HUMPHREYS, S. Tryptophane derivatives in urine of pyridoxine-deficient swine, *Bull. Johns Hopkins Hosp.*, **75**:35-47, 1944.
 30. CEDER, E. T., and ZON, L. The treatment of psoriasis with massive doses of crystalline vitamin D and irradiated ergosterol; preliminary report, *Pub. Health Rep.*, **52**:1580-84, 1937.
 31. CHAMBERS, R., and ZWEIFACH, B. W. Capillary endothelial cement in relation to

- permeability, *J. Cell. & Comp. Physiol.*, **15**:255-72, 1940.
32. CHARPY, M. J. À propos du procès-verbal "À propos du traitement du lupus de Willan par la méthode de Charpy," *Ann. de dermat. et syph.*, **4**:331-32, 1944.
 33. CHITTENDEN, R. H., and UNDERHILL, F. P. The production in dogs of a pathological condition which closely resembles human pellagra, *Am. J. Physiol.*, **44**:13-66, 1917.
 34. CLINE, J. K.; WILLIAMS, R. R.; and FINKELSTEIN, J. Studies of crystalline vitamin B₁. XVIII. Synthesis of vitamin B₁, *J. Am. Chem. Soc.*, **59**:1052-54, 1937.
 35. CLOETENS, R. Reversible Abspaltung des zweiten Metalles der alkalischen Phosphatase. II, *Biochem. Ztschr.*, **308**:37-39, 1941.
 36. CONNERS, C. A.; ECKHARDT, R. E.; and JOHNSON, L. V. Riboflavin for rosacea keratitis, marginal corneal ulcers and catarrhal corneal infiltrates; laboratory and clinical studies, *Arch. Ophth.*, **29**:956-67, 1943.
 37. COOPERMAN, J. M.; WAISMAN, H. A.; and ELVEHJEM, C. A. Nutrition of the golden hamster, *Proc. Soc. Exper. Biol. & Med.*, **52**:250-54, 1943.
 38. CORNBLEET, T., and PACE, E. R. Use of maize oil (unsaturated fatty acids) in the treatment of eczema, *Arch. Dermat. & Syph.*, **31**:224-26, 1935.
 39. CORNBLEET, T., and STRUCK, H. C. Calcium metabolism in scleroderma, *Arch. Dermat. & Syph.*, **35**:188-201, 1937.
 40. CURTIS, A. C.; TAYLOR, H.; and GREKIN, R. H. Sarcoidosis. I. Results of treatment with varying amounts of calciferol and dihydrotachysterol, *J. Invest. Dermat.*, **9**:131-50, 1947.
 41. DAFT, F. S., and SEBRELL, W. H. The successful treatment of granulocytopenia and leukopenia in rats with crystalline folic acid, *Pub. Health Rep.*, **58**:1542-45, 1943.
 42. DAM, H. Vitamin K, its discovery, biochemistry and application in medicine (Edward Gamaliel Janeway lecture), *J. Mt. Sinai Hosp.*, **12**:961-70, 1946.
 43. DANN, W. J., and HANDLER, P. The nicotinic acid and coenzyme content of the tissues of normal and blacktongue dogs, *J. Nutrition*, **22**:409-14, 1941.
 44. DAY, H. G. The effects of zinc deficiency in the mouse, *Federation Proc.*, **1**:188-89, 1942.
 45. DAY, H. G., and MCCOLLUM, E. V. Effects of acute dietary zinc deficiency in the rat, *Proc. Soc. Exper. Biol. & Med.*, **45**:282-84, 1940.
 46. DAY, P. L.; MIMS, V.; and TOTTER, J. R. The relationship between vitamin M and the *L. casei* factor, *J. Biol. Chem.*, **161**:45-52, 1945.
 47. DOWLING, G. B., and THOMAS, E. W. P. Lupus vulgaris treated with calciferol, *Proc. Roy. Soc. Med.*, **39**:96-99, 1945.
 48. DU VIGNEAUD, V.; MELVILLE, D. B.; GYÖRGY, P.; and ROSE, C. S. On the identity of vitamin H with biotin, *Science*, **92**:62-63, 1940.
 49. EAKIN, R. E.; SNELL, E. E.; and WILLIAMS, R. J. The concentration and assay of avidin, the injury producing protein in raw egg white, *J. Biol. Chem.*, **140**:535-43, 1941.
 50. EBERT, M. H. Vitamins in dermatology, *M. Clin. North America*, **26**:47-64, 1942.
 51. ELVEHJEM, C. A.; MADDEN, R. J.; STRONG, F. M.; and WOOLLEY, D. W. Relation of nicotinic acid and nicotinic acid amide to canine blacktongue, *J. Am. Chem. Soc.*, **59**:1767-68, 1937.
 52. ELVEHJEM, C. A.; MCINTYRE, J. M.; and SCHWEIGERT, B. S. The nutrition of the cotton rat, *J. Nutrition*, **27**:1-9, 1944.
 53. ENGEL, R. W. The relation of B vitamins and dietary fat to the lipotropic action of choline, *J. Nutrition*, **24**:175-85, 1942.
 54. EPSTEIN, N. N., and GLICK, D. Unsaturated fatty acids in eczema; observations on acne vulgaris, psoriasis, xanthoma tuberosum and xanthoma palpebrarum, *Arch. Dermat. & Syph.*, **35**:427-32, 1937.
 55. EULER, H. VON; ALBERS, H.; and SCHLENCK, F. Über die Cozymase, *Ztschr. f. physiol. Chem.*, **237**:1-11, 1935.
 56. EVANS, H. M.; EMERSON, O. H.; and EMERSON, G. A. The isolation from wheat germ oil of an alcohol, α -tocopherol, having the properties of vitamin E, *J. Biol. Chem.*, **113**:319-32, 1936.
 57. EVERETT, M. R. Medical biochemistry. 2d ed. New York: Paul B. Hoeber, Inc., 1946.
 58. FINNERUD, C. W.; KESLER, R. L.; and WIESE, H. F. Ingestion of lard in treatment of eczema and allied dermatoses;

- clinical and biochemical study, *Arch. Dermat. & Syph.*, **44**:849-61, 1941.
59. FITZPATRICK, T. B. Personal communication.
60. FOLLIS, R. H., JR. The pathology of nutritional disease. Springfield, Ill: Charles C Thomas, 1948.
61. FOLLIS, R. H., JR.; DAY, H. G.; and MCCOLLUM, E. V. Histological studies of the tissues of rats fed a diet extremely low in zinc, *J. Nutrition*, **22**:223-37, 1941.
62. FRAZIER, C. N., and HU, C. K. Cutaneous lesions associated with vitamin A deficiency in man, *Arch. Int. Med.*, **48**:507-14, 1931.
63. ———. Nature and distribution according to age of cutaneous manifestations of vitamin A deficiency: a study of two hundred and seven cases, *Arch. Dermat. & Syph.*, **33**:825-52, 1936.
64. FRAZIER, C. N.; HU, C. K.; and CHU, F. T. Variations in the cutaneous manifestations of vitamin A deficiency from infancy to puberty, *Arch. Dermat. & Syph.*, **48**:1-14, 1943.
65. FRAZIER, C. N., and LI, H. C. Vitamin A deficiency in man: resolution of cutaneous lesions following parenteral administration of carotene, *Chinese M. J.*, **54**:301-14, 1938.
66. FRAZIER, C. N., and MARMELSZADT, W. L. Vitamin A deficiency and the skin in retrospect. An historical search into nosological antecedents, *Bull. Hist. Med.*, **22**:766-95, 1948.
67. FRAZIER, C. N., and TSAO, S. N. Association of neuromuscular symptoms and cutaneous lesions in vitamin A deficiency: a discussion of the etiologic relationship, *Chinese M. J.*, **59**:399-415, 1941.
68. FRICKE, H. H.; LANIUS, B.; DE ROSE, A. F.; LAPIDUS, M.; and FROST, D. V. Comparison of vitamin B₁₂ and vitamin B_{12b}, *Federation Proc.*, **9**:173, 1950.
69. FUNK, C. On the chemical nature of the substance which cures polyneuritis in birds induced by a diet of polished rice, *J. Physiol.*, **43**:395-400, 1911.
70. GAVIN, G., and MCHENRY, E. W. Inositol: a lipotropic factor, *J. Biol. Chem.*, **139**:485, 1941.
71. GEIGER, E. Experiments with delayed supplementation of incomplete amino-acid mixtures, *J. Nutrition*, **34**:97-111, 1947.
72. GINSBERG, J. E.; BERNSTEIN, C., JR.; and IOB, L. V. Effect of oils containing unsaturated fatty acids on patients with dermatitis, with brief report of experimental study in guinea pigs, *Arch. Dermat. & Syph.*, **36**:1033-38, 1937.
73. GOLDBERGER, J., and WHEELER, G. A. Experimental blacktongue of dogs and its relation to pellagra, *Pub. Health Rep.*, **43**:172-217, 1928.
74. GOLDBLUM, R. W.; DERBY, S.; and LERNER, A. B. The metal content of skin, nails and hair, *J. Invest. Dermat.*, **20**:13-18, 1953.
75. GORDON, E. S., and SEVRINGHAUS, E. L. Vitamin therapy in general practice. Chicago: Year Book Publishers, 1942.
76. GORRIE, D. R. Purpura haemorrhagica after arsenic therapy treated with vitamin P, *Lancet*, **1**:1005-7, 1940.
77. GREENBERG, D. M. Studies in mineral metabolism with the aid of artificial radioactive isotopes. VIII. Tracer experiments with radioactive calcium and strontium on the mechanism of vitamin D action in rachitic rats, *J. Biol. Chem.*, **157**:99-104, 1945.
78. GROSS, P. Role of unsaturated fatty acids in acrodynia (vitamin B₆ deficiency) of the rat, *J. Invest. Dermat.*, **3**:505-22, 1940.
79. ———. Nonpellagrous eruptions due to deficiency of vitamin B complex, *Arch. Dermat. & Syph.*, **43**:504-31, 1941.
80. GROSS, P.; ZABOJ, H.; and RUNNE, E. Vitamin deficiency syndrome in the albino rat precipitated by chronic zinc chloride poisoning, *J. Invest. Dermat.*, **4**:385-98, 1941.
81. GUBNER, R. Therapeutic suppression of tissue reactivity; comparison of effects of cortisone and aminopterin, *Am. J. M. Sc.*, **221**:169-75, 176, 1951.
82. GYÖRGY, P. Vitamin B₂ and pellagra-like dermatitis in rats, *Nature*, **133**:498-99, 1934.
83. ———. Investigation on the vitamin B₂ complex. 1. The differentiation of lactoflavin and the "rat anti-pellagra" factor, *Biochem. J.*, **29**:741-59, 1935.
84. ———. The curative factor (vitamin H) for egg white injury, with particular reference to its presence in different foodstuffs and in yeast, *J. Biol. Chem.*, **131**:733-44, 1939.
85. ———. Dietary treatment of scalp desquamative dermatoses of seborrheic type;

- experimental foundation, *Arch. Dermat. & Syph.*, **43**:230-47, 1941.
86. GYÖRGY, P., and ECKHARDT, R. E. Vitamin B₆ and skin lesions in rats, *Nature*, **144**:512, 1939.
 87. HANDLER, P. Use of highly purified rations in the study of nicotinic acid deficiency, *Proc. Soc. Exper. Biol. & Med.*, **52**:263-64, 1943.
 88. ———. Factors affecting the occurrence of hemorrhagic kidneys due to choline deficiency, *J. Nutrition*, **31**:621-33, 1946.
 89. HANSEN, A. E. Serum lipids in eczema and in other pathologic conditions, *Am. J. Dis. Child.*, **53**:933-46, 1937.
 90. HANSEN, A. E., and WIESE, H. F. Studies with dogs maintained on diets low in fat, *Proc. Soc. Exper. Biol. & Med.*, **52**:205-8, 1943.
 91. HARRIS, S. A., and FOLKERS, K. Synthetic vitamin B₆, *Science*, **89**:347, 1939.
 92. HARRIS, S. A.; WOLF, D. E.; MOZINGO, R.; and FOLKERS, K. Synthetic biotin, *Science*, **97**:447-48, 1943.
 93. HART, E. B.; STEENBOCK, H.; WADDELL, J.; and ELVEHJEM, C. A. Iron in nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat, *J. Biol. Chem.*, **77**:797-812, 1928.
 94. HEARD, E. V., and LEWIS, H. B. The metabolism of sulfur. XXV. Dietary methionine as a factor related to the growth and composition of the hair of the young white rat, *J. Biol. Chem.*, **123**:203-10, 1938.
 95. HEIDELBERGER, C.; ABRAHAM, E. P.; and LEPKOVSKY, S. Tryptophane metabolism. II. Concerning the mechanism of the mammalian conversion of tryptophane into nicotinic acid, *J. Biol. Chem.*, **179**:151-55, 1949.
 96. HESS, A. F., and WEINSTOCK, M. Anti-rachitic properties imparted to inert fluids and to green vegetables by ultra-violet irradiation, *J. Biol. Chem.*, **62**:301-13, 1924.
 97. HIRST, E. L. The structure of ascorbic acid, *Chem. & Indust.*, pp. 221-22, 1933.
 98. HOLMBERG, C. G. Uricase purification and properties, *Biochem. J.*, **33**:1901-6, 1939.
 99. HOOKER, C. W., and PFEIFFER, C. A. Effects of sex hormones upon body growth, skin, hair, and sebaceous glands in the rat, *Endocrinology*, **32**:69-76, 1943.
 100. HORWITT, M. K.; HILLS, O. W.; HARVEY, C. C.; LIEBERT, E.; and STEINBERG, D. L. Effects of dietary depletion of riboflavin, *J. Nutrition*, **39**:357-73, 1949; also *Arch. Int. Med.*, **87**:682-93, 1951.
 101. HOVE, E.; ELVEHJEM, C. A.; and HART, E. B. The physiology of zinc in the nutrition of the rat, *Am. J. Physiol.*, **119**:768-75, 1937.
 102. ———. Further studies on zinc deficiency in rats, *ibid.*, **124**:750-58, 1938.
 103. ———. The effect of zinc on alkaline phosphatases, *J. Biol. Chem.*, **134**:425-42, 1940.
 104. ———. The relation of zinc to carbonic anhydrase, *ibid.*, **136**:425-34, 1940.
 105. HUNDLEY, J. M., and ING, R. B. Effect of pantothenic acid deficiency on skin copper, *Federation Proc.*, **10**:385, 1951.
 106. HUNT, C. H. Unpublished paper, given at Gibson Island Research Conf. AAAS, July, 1940. Quoted by MORGAN, A. F., The effect of vitamin deficiencies on adrenocortical function. *In*: HARRIS, R. S., and THIMANN, K. V. (eds.), *Vitamins and hormones*, **9**:161-212. New York: Academic Press, Inc., 1951.
 107. JERSILD, T. Therapeutic effect of vitamin P in Schönlein-Henoch purpura, *Lancet*, **1**:1445-47, 1938.
 108. JERVIS, G. A. Phenyl pyruvic oligophrenia. Introductory study of 50 cases of mental deficiency associated with excretion of phenylpyruvic acid, *Arch. Neurol. & Psychiat.*, **38**:944-63, 1937.
 109. JOHNSON, H. H., JR., and BINCKLEY, G. W. Nicotinic acid therapy of dermatitis herpetiformis, *J. Invest. Dermat.*, **14**:233-38, 1950.
 110. JOLLIFFE, N.; ROSENBLUM, L. A.; and SAWHILL, J. Effects of pyridoxine (vitamin B₆) on persistent adolescent acne, *J. Invest. Dermat.*, **5**:143-48, 1942.
 111. JUKES, T. H., and STOKSTAD, E. L. R. The role of vitamin B₁₂ in metabolic processes. *In*: HARRIS, R. S., and THIMANN, K. V. (eds.), *Vitamins and hormones*, **9**:1-26. New York: Academic Press, Inc., 1951.
 112. KACZKA, E. A.; DENKEWALTER, R. G.; HOLLAND, A.; and FOLKERS, K. Vitamin B₁₂. XIII. Additional data on vitamin B_{12a}, *J. Am. Chem. Soc.*, **73**:335-37, 1951.
 113. KACZKA, E. A.; WOLF, D. E.; and FOLKERS, K. Vitamin B₁₂. V. Identification of

- crystalline vitamin B_{12a}, J. Am. Chem. Soc., **71**:1514-15, 1949.
114. KACZKA, E. A.; WOLF, D. E.; KUEHL, F. A., JR.; and FOLKERS, K. Vitamin B₁₂: reactions of cyano-cobalamin and related compounds, Science, **112**:354-55, 1950.
115. KARK, R.; SOUTER, A. W.; and HAYWARD, J. C. Haemorrhagic diathesis in idiopathic steatorrhoea; observations on its association with vitamin K deficiency, Quart. J. Med., **9**:247-61, 1940.
116. KARRER, P.; FRITZSCHE, H.; RINGIER, B. H.; and SOLOMON, H. α -Tocopherol, Helvet. chim. acta, **21**:520-25, 1938.
117. KARRER, P.; HELFENSTEIN, A.; WEHRLI, H.; and WETTSTEIN, A. Pflanzenfarbstoffe. XXV. Ueber die Konstitution des Lycopens und Carotins, Helvet. chim. acta, **13**:1084-99, 1930.
118. KARRER, P.; MORF, R.; and SCHÖPP, K. Zur Kenntnis des vitamins-A aus Fischtranen. II, Helvet. chim. acta, **14**:1431-36, 1931.
119. KEILIN, D., and MANN, T. Carbonic anhydrase, Nature, **144**:442-43, 1939.
120. KERESZTESY, J. C., and STEVENS, J. R. Crystalline vitamin B₆, Proc. Soc. Exper. Biol. & Med., **38**:64-65, 1938.
121. KING, C. G., and WAUGH, W. A. Chemical nature of vitamin C, Science, **75**:357-58, 1932.
122. KLAUDER, J. V., and BROWN, H. Experimental studies in eczema; correlation of potassium-calcium ratio in serum and in skin of rabbits with irritability of skin, Arch. Dermat. & Syph., **20**:326-34, 1929.
123. KORNBERG, A.; DAFT, F. S.; and SEBRELL, W. H. Production of vitamin K deficiency in rats by various sulfonamides, Pub. Health Rep., **59**:832-44, 1944.
124. KORNBERG, A.; TABOR, H.; and SEBRELL, W. H. Blood regeneration in pyridoxine-deficient rats, Am. J. Physiol., **143**:434-39, 1945.
125. KRAFKA, J., JR. Vitamin D therapy in psoriasis, J. M. A. Georgia, **30**:398-400, 1941.
126. KREHL, W. A.; TEPLY, L. J.; SARMA, P. S.; and ELVEHJEM, C. A. Growth-retarding effect of corn in nicotinic acid-low rations and its counteraction by tryptophane, Science, **101**:489-90, 1945.
127. KRUSE, H. D.; ORENT, E.; and MCCOLLUM, E. V. Studies on magnesium in animals. I. Symptomatology resulting from magnesium deficiency, J. Biol. Chem., **96**:519-39, 1932.
128. KUGELMASS, I. N. Vitamin P in vascular purpura, J.A.M.A., **115**:519-20, 1940.
129. KUHN, R.; GYÖRGY, P.; and WAGNER-JAUREGG, T. Über ein neue Klasse von Naturfarbstoffen, Ber. d. deutsch. chem. Gesellsch., **66**:317-20, 1933.
130. KUHN, R.; REINEMUND, K.; WEYGAND, F.; and STRÖBELE, R. Über die Synthese des Lactoflavins, Ber. d. deutsch. chem. Gesellsch., **68**:1765-74, 1935.
131. LEASE, J. G.; PARSONS, H. T.; and KELLY, E. A comparison in five types of animals of the effects of dietary egg white and of a specific factor given orally or parenterally, Biochem. J., **31**:433-37, 1937.
132. LEE, T. H.; LERNER, A. B.; and HALBERG, R. J. Water soluble vitamins in normal human skin, J. Invest. Dermat., **20**:19-26, 1953.
133. LEPKOVSKY, S., and NIELSEN, E. A green pigment-producing compound in urine of pyridoxine-deficient rats, J. Biol. Chem., **144**:135-38, 1942.
134. LEPKOVSKY, S.; ROBOZ, E.; and HAAGEN-SMIT, A. J. Xanthurenic acid and its role in tryptophane metabolism of pyridoxine-deficient rats, J. Biol. Chem., **149**:195-201, 1943.
135. LEVER, W. F., and TALBOTT, J. H. Pemphigus; further report on chemical studies of blood serum and treatment with adrenocortical extract, dihydrotachysterol or vitamin D, New England J. Med., **231**:44-51, 1944.
136. LEVINE, S. Z.; GORDON, H. H.; and MARPLES, E. A defect in the metabolism of tyrosine and phenylalanine in premature infants. II. Spontaneous occurrence and eradication by vitamin C, J. Clin. Investigation, **20**:209-19, 1941.
137. LICHSTEIN, H. C. Functions of biotin in enzyme systems. In: HARRIS, R. S., and THIMANN, K. V. (eds.), Vitamins and hormones, **9**:27-74. New York: Academic Press, Inc., 1951.
138. LINDHEIMER, G. T.; HINMAN, W. F.; and HALLIDAY, E. G. Function and occurrence of citrin ("vitamin P"), J. Am. Dietet. A., **18**:503-7, 1942.
139. LIPMANN, F.; KAPLAN, N. D.; NOVELLI, G. D.; TUTTLE, L. C.; and GUIRARD, B. M. Coenzyme for acetylation, a pantothenic

- acid derivative, *J. Biol. Chem.*, **167**:869-70, 1947.
140. LOHMANN, K., and SCHUSTER, P. Untersuchungen über die Carboxylase, *Biochem. Ztschr.*, **294**:188-214, 1937.
 141. LUITHLEN, F. Das gegenseitige Kationenverhältnis bei verschiedener Ernährung und bei Säurevergiftung, *Arch. f. exper. Path. u. Pharmacol.*, **68**:209-30, 1912.
 142. MCCALL, K. B.; WAISMAN, H. A.; ELVEHJEM, C. A.; and JONES, E. S. A study of pyridoxine and pantothenic acid deficiencies in the monkey (*Macaca mulatta*), *J. Nutrition*, **31**:685-97, 1946.
 143. MACCARDLE, R. C.; ENGMAN, M. F., JR.; and ENGMAN, F. M. Spectrographic analysis of neurodermatitic lesions; human magnesium deficiency, *Arch. Dermat. & Syph.*, **44**:429-40, 1941.
 144. ———. Mineral changes in neurodermatitis revealed by microincineration, *ibid.*, **47**:335-72, 1943.
 145. MCCOLLUM, E. V., and DAVIS, M. The necessity of certain lipins in the diet during growth, *J. Biol. Chem.*, **15**:167-75, 1913.
 146. ———. The nature of the dietary deficiencies of rice, *ibid.*, **23**:181-230, 1915.
 147. MCCOLLUM, E. V., and ORENT, E. R. Effects on the rat of deprivation of magnesium, *J. Biol. Chem.*, **92**:xxx, 1931.
 148. MCCONNELL, R. B., and CHEETHAM, H. D. Acute pellagra during isoniazid therapy, *Lancet*, **2**:959-60, 1952.
 149. MCELROY, L. W.; SALMON, K.; FIGGE, F. H. J.; and COWGILL, G. R. On the porphyrin nature of the fluorescent "blood caked" whiskers of pantothenic acid deficient rats, *Science*, **94**:467, 1941.
 150. MARSTON, H. R. Cobalt, copper and molybdenum in the nutrition of animals and plants, *Physiol. Rev.*, **32**:66-121, 1952.
 151. MARTIN, G. J. Calcium deficiency syndrome produced in growing animals, *Growth*, **1**:175-81, 1937.
 152. MASKILLEYSON, L. N.; BENYAMOVICH, E. B.; KRICHEVSKAYA, E. D.; and SHATAMOVA, L. V. Role of vitamins in pathogenesis and treatment of skin diseases, *Am. Rev. Soviet Med.*, **3**:19-27, 1945.
 153. MILBRADT, W. Experimentelle Untersuchungen zur Frage der Bedeutung des H-Vitamins für die menschliche Pathologie, *Dermat. Wchnschr.*, **103**:1376 and 1402, 1936.
 154. MILLER, A. A. Purpura in course of measles: case treated with vitamin P, *Brit. J. Child. Dis.*, **38**:1-14, 1941.
 155. MINER, D. L.; MILLER, J. A.; BAUMANN, C. A.; and RUSCH, H. P. The effect of pyridoxine and other B vitamins on the production of liver cancer with *p*-dimethylaminoazobenzene, *Cancer Research*, **3**:296-302, 1943.
 156. MITCHELL, H. K.; SNELL, E. E.; and WILLIAMS, R. J. Concentration of "folic acid," *J. Am. Chem. Soc.*, **63**:2284, 1941.
 157. MONCORPS, C. Zur Frage des Vitamin H, *Dermat. Wchnschr.*, **103**:1230-31, 1936.
 158. MONTAGNA, W. Effect of biotin deficiency upon the skin of mice, *Proc. Soc. Exper. Biol. & Med.*, **73**:127-31, 1950.
 159. MONTGOMERY, M. L.; SHELIN, G. E.; and CHAIKOFF, I. L. The elimination of administered zinc in pancreatic juice, duodenal juice and bile of dogs as measured by its radioactive isotope (Zn^{65}), *J. Exper. Med.*, **78**:151-59, 1943.
 160. MOORE, R. A.; BITTINGER, I.; MILLER, M. L.; and HELLMAN, L. M. Abortion in rabbits fed a vitamin K deficient diet, *Am. J. Obst. & Gynec.*, **43**:1007-12, 1942.
 161. MOORE, T. Vitamin A and carotene. VI. The conversion of carotene to vitamin A in vivo, *Biochem. J.*, **24**:692-702, 1930.
 162. MORRIS, J. E.; HARPUR, E. R.; and GOLDBLOOM, A. The metabolism of *l*-tyrosine in infantile scurvy, *J. Clin. Investigation*, **29**:325-35, 1950.
 163. NELSON, C. T. Calciferol in the treatment of sarcoidosis, *J. Invest. Dermat.*, **13**:81-84, 1943.
 164. NICOLAYSEN, R. Studies on the mode of action of vitamin D. III. The influence of vitamin D on the absorption of calcium and phosphorus in the rat, *Biochem. J.*, **31**:122-29, 1937.
 165. NIELSEN, E., and BLACK, A. Biotin and folic acid deficiencies in the mouse, *J. Nutrition*, **28**:203-6, 1944.
 166. NOVAK, L. J., and BERGEIM, O. Water soluble vitamins in hair as influenced by diet, *J. Biol. Chem.*, **155**:283-90, 1944.
 167. OLCOTT, H. S., and MCCANN, D. C. Carotenase. The transformation of carotene into vitamin A in vitro, *J. Biol. Chem.*, **94**:185-93, 1931.
 168. ORENT, E.; KRUSE, H. D.; and MCCOL-

- LUM, E. V. Studies on magnesium deficiency in animals. II. Species variation in symptomatology of magnesium deprivation, *Am. J. Physiol.*, **101**:454-61, 1932.
169. OSBORNE, T. B., and MENDEL, L. B. The relation of growth to the chemical constituents of the diet, *J. Biol. Chem.*, **15**:311-26, 1913.
170. OVERMAN, R. S.; FIELD, J. B.; BAUMANN, C. A.; and LINK, K. P. Studies on the hemorrhagic sweet clover disease. IX. The effect of diet and vitamin K on the hypoprothrombinemia induced by 3,3'-methylenebis(4-hydroxycoumarin) in the rat, *J. Nutrition*, **23**:589-602, 1942.
171. PEACOCK, S. C., and KNOWLTON, K. Alkaptonuria, *Am. J. Dis. Child.*, **56**:100-102, 1938.
172. PENNINGTON, D.; SNELL, E. E.; and EAKIN, R. E. Crystalline avidin, *J. Am. Chem. Soc.*, **64**:469, 1942.
173. PEREZ, W. M.; SOFFER, L. J.; and SILBERT, S. Improvement in two cases of diffuse scleroderma by use of dihydro-tachysterol (A.T. 10); preliminary report, *J. Mt. Sinai Hosp.*, **6**:333-37, 1940.
174. PETERS, R. A. The biochemical lesion in vitamin B deficiency; application of modern biochemical analysis in its diagnosis, *Lancet*, **1**:1161-65, 1936.
175. PHILPOT, F. J., and PIRIE, A. Riboflavin and riboflavin adenine dinucleotide in ox ocular tissue, *Biochem. J.*, **37**:250-54, 1943.
176. PIERCE, J. V.; PAGE, A. C., JR.; STOKSTAD, E. L. R.; and JUKES, T. H. Crystallization of vitamin B_{12b}, *J. Am. Chem. Soc.*, **71**:2952, 1949.
177. QUACKENBUSH, F. W.; PLATZ, B. R.; and STEENBOCK, H. Rat acrodynia and the essential fatty acids, *J. Nutrition*, **17**:115-26, 1939.
178. RAUCH, H. The effects of biotin deficiency on hair development and pigmentation, *Physiol. Zool.*, **25**:145-49, 1952.
179. REICHSTEIN, T.; GRÜSSNER, A.; and OPPENAWER, R. Synthesis of *d*- and *l*-ascorbic acid (vitamin C), *Helvet. chim. acta*, **16**:1019-33, 1933.
180. REID, D. F.; LEPKOVSKY, S.; BONNER, O.; and TATUM, E. L. The intermediary metabolism of tryptophane in pyridoxine deficient rats, *J. Biol. Chem.*, **155**:299-303, 1944.
181. RICKES, E. L.; BRINK, N. G.; KONIUSZY, F. R.; WOOD, T. R.; and FOLKERS, K. Crystalline vitamin B₁₂, *Science*, **107**:396-97, 1948.
182. ROBERTSON, R. F. Vitamin D in Boeck's sarcoidosis, *Brit. M. J.*, **2**:1059-61, 1948.
183. ROSE, W. C. Amino-acid requirements of man, *Federation Proc.*, **8**:546-52, 1949.
184. ROSEN, F.; HUFF, J. W.; and PERLZWEIG, W. A. The effect of tryptophane on the synthesis of nicotinic acid in the rat, *J. Biol. Chem.*, **163**:343-44, 1946.
185. ROTHMAN, S., and FLESCH, P. The physiology of the skin, *Ann. Rev. Physiol.*, **6**:195-224, 1944.
186. ROUTH, J. L., and HOUCHIN, O. B. Some nutritional requirements of the hamster, *Federation Proc.*, **1**:191-92, 1942.
187. RUSZNYAK, S., and SZENT-GYÖRGYI, A. Vitamin P: flavanols as vitamins, *Nature*, **138**:27, 1936.
188. SALMON, W. D. The supplementary relationship of vitamin B₆ and unsaturated fatty acids, *J. Biol. Chem.*, **133**:lxxxiii, 1940.
189. SCARBOROUGH, H. Deficiency of vitamin C and vitamin P in man, *Lancet*, **2**:644-47, 1940.
190. ———. Discussion on vitamins and haemorrhagic states, *Proc. Roy. Soc. Med.*, **35**:407-10, 1942.
191. SCHNEIDER, H.; STEENBOCK, H.; and PLATZ, B. R. Essential fatty acids, vitamin B₆, and other factors in the cure of rat acrodynia, *J. Biol. Chem.*, **132**:539-51, 1940.
192. SCHOENHEIMER, R., and RITTENBERG, D. The study of intermediary metabolism of animals with the aid of isotopes, *Physiol. Rev.*, **20**:218-48, 1940.
193. SCHREINER, A. W.; SLINGER, W.; HAWKINS, V. R.; and VILTER, R. W. Seborrheic dermatitis, a local metabolic defect involving pyridoxine, *J. Lab. & Clin. Med.*, **40**:121-30, 1952.
194. SCHWEMMLER, B. Einfluss von Vitamin A auf Haar- und Nagelwachstum, *München. med. Wchnschr.*, **86**:1126-1227, 1939.
195. SEALOCK, R. R., and SILBERSTEIN, H. E. The excretion of homogentisic acid and other tyrosine metabolites by the vitamin C-deficient guinea pig, *J. Biol. Chem.*, **135**:251-58, 1940.
196. SEBRELL, W. H., and BUTLER, R. E. Riboflavin deficiency in man. Preliminary note, *Pub. Health Rep.*, **53**:2282-84, 1938.

197. SHELINE, G. E.; CHAIKOFF, I. L.; JONES, H. B.; and MONTGOMERY, M. L. Studies on the metabolism of zinc with the aid of its radioactive isotope. I. The excretion of zinc in the urine and feces, *J. Biol. Chem.*, **147**:409-14, 1943.
198. ———. Studies on the metabolism of zinc with the aid of its radioactive isotope. II. The distribution of administered radioactive zinc in the tissues of mice and dogs, *ibid.*, **149**:139-51, 1943.
199. SHUTE, E. V.; VOGELSANG, A. B.; SKELTON, F. R.; and SHUTE, W. E. The influence of vitamin E on vascular disease, *Surg., Gynec. & Obst.*, **86**:1-8, 1948.
200. SILBER, R. H. Studies of pantothenic acid deficiency in dogs, *J. Nutrition*, **27**:425-33, 1944.
201. SINGER, L., and DAVIS, G. K. Pantothenic acid in copper deficiency in rats, *Science*, **111**:472-73, 1950.
202. SINGHER, H. O.; KENSLE, C. J.; TAYLOR, H. C.; RHOADS, C. P.; and UNNA, K. The effect of vitamin deficiency on estradiol inactivation by liver, *J. Biol. Chem.*, **154**:79-86, 1944.
203. SJÖGREN, H. Zur Kenntnis der Keratoconjunctivitis sicca; allgemeine Symptomatologie und Ätiologie, *Acta ophth.*, **13**:1-39, 1935.
204. SMITH, S. G., and MARTIN, D. W. Cheilosis successfully treated with synthetic vitamin B₆, *Proc. Soc. Exper. Biol. & Med.*, **43**:660-63, 1940.
205. SMITH, E. L., and PARKER, L. F. J. Purification of anti-pernicious anaemia factor, *Biochem. J.*, **43**:viii, 1948.
206. SMITH, H. P.; WARNER, E. D.; BRINKHOUS, K. M.; and SEEGER, W. H. Bleeding tendency and prothrombin deficiency in biliary fistula dogs, *J. Exper. Med.*, **67**:911-20, 1938.
207. SMUTS, D. B.; MITCHELL, H. H.; and HAMILTON, T. S. The relation between dietary cystine and the growth and cystine content of hair in the rat, *J. Biol. Chem.*, **95**:283-95, 1932.
208. SPIES, T. D.; BEAN, W. B.; and ASHE, W. F. A note on the use of vitamin B₆ in human nutrition, *J.A.M.A.*, **112**:2414-15, 1939.
209. SPIES, T. D.; HIGHTOWER, D. P.; and HUBBARD, L. H. Some recent advances in vitamin therapy, *J.A.M.A.*, **115**:292-97, 1940.
210. STAHEL, W. Das Sjögrensche Syndrom eine A-Hypo-Vitaminose, *Klin. Wchnschr.*, **17**:1692-94, 1938.
211. STEENBOCK, H. White corn vs. yellow corn as a probable relation between the fat soluble vitamin and yellow plant pigments, *Science*, **50**:352, 1919.
212. STEENBOCK, H., and BLACK, A. Fat soluble vitamins. XVII. The induction of growth-promoting and calcifying properties in a ration by exposure to ultra-violet light, *J. Biol. Chem.*, **61**:405-22, 1924.
213. STILLER, E. T.; KERESZTESY, J. C.; and STEVENS, J. R. The structure of vitamin B₆, *J. Am. Chem. Soc.*, **61**:1237-42, 1939.
214. SULLIVAN, M., and EVANS, V. J. Nutritional dermatoses in the rat. IX. Evaluation of the interrelationship of magnesium deficiency and deficiencies of the vitamin B complex, *J. Nutrition*, **27**:123-35, 1944.
215. ———. Nutritional dermatoses in the rat. X. A comparison of disseminated neurodermatitis and experimental magnesium deficiency, *Arch. Dermat. & Syph.*, **49**:33-45, 1944.
216. ———. Nutritional dermatoses in the rat. XI. Vitamin A deficiency superimposed on vitamin B complex deficiency, *ibid.*, **51**:17-25, 1945.
217. SULLIVAN, M.; KOLB, L. C.; and NICHOLLS, J. Nutritional dermatoses in the rat. VII. Notes on the posture, gait, and hypertonicity resulting from a diet containing unheated, dried egg white as the source of protein, *Bull. Johns Hopkins Hosp.*, **70**:177-85, 1942.
218. SULLIVAN, M., and NICHOLLS, J. Nutritional approach to experimental dermatology. Nutritional dermatoses in the rat. I. Vitamin B₆ deficiency, *J. Invest. Dermat.*, **3**:317-35, 1940.
219. ———. Nutritional dermatoses in the rat. IV. Riboflavin deficiency, *ibid.*, **4**:181-91, 1941.
220. ———. Nutritional dermatoses in the rat. V. Signs and symptoms resulting from a diet containing unheated, dried egg white as the source of protein, *Arch. Dermat. & Syph.*, **45**:295-314, 1942.
221. ———. Nutritional dermatoses in the rat. VI. The effect of pantothenic acid deficiency, *ibid.*, pp. 917-32.
222. SYDENSTRICKER, V. P.; SINGAL, S. A.;

- BRIGGS, A. P.; DE VAUGHN, N. M.; and ISBELL, H. Observations on the "egg white injury" in man and its cure with a biotin concentrate, *J.A.M.A.*, **118**:1199-1200, 1942.
223. SZENT-GYÖRGYI, A. Observations on the functions of the peroxidase systems and the chemistry of the adrenal cortex. Description of a new carbohydrate derivative, *Biochem. J.*, **22**:1387-1409, 1928.
224. TAUB, S. J., and ZAKON, S. J. The use of unsaturated fatty acids in the treatment of eczema (atopic dermatitis, neurodermatitis), *J.A.M.A.*, **105**:1675, 1935.
225. TAUBER, E. B., and CLARKE, G. E. Treatment of pemphigus with concentrated viosterol, *Arch. Dermat. & Syph.*, **40**:82-89, 1939.
226. THÉLIN, F. Dermatite séborrhoïde et biotine, *Ann. paediat.*, **172**:193-97, 1949.
227. TISDALL, F. F.; MCCREARY, J. F.; and PEARCE, H. The effect of riboflavin on corneal vascularization and symptoms of eye fatigue in R.C.A.F. personnel, *Canad. M. A. J.*, **49**:5-13, 1943.
228. TODD, W. R.; ELVEHJEM, C. A.; and HART, E. B. Zinc in the nutrition of the rat, *Am. J. Physiol.*, **107**:146-56, 1934.
229. TUFTS, E. V., and GREENBERG, D. M. Nature of magnesium tetany, *Am. J. Physiol.*, **121**:416-23, 1938.
230. URBACH, E. Skin diseases, nutrition and metabolism. New York: Grune & Stratton, 1946.
231. VEER, W. L. C.; EDELHAUSEN, J. H.; WIGMENG, H. G.; and LENS, J. Vitamin B₁₂. I. The relation between vitamin B₁₂ and vitamin B_{12b}, *Biochim. et biophys. acta*, **6**:225-28, 1950.
232. VILTER, R. W.; HERRIGAN, D.; MUELLER, J. F.; JARROLD, T.; VILTER, C. F.; HAWKINS, V.; and SEAMAN, A. Studies on the relationships of vitamin B₁₂, folic acid, thymine, uracil, and methyl group donors in persons with pernicious anemia and related megaloblastic anemias, *Blood*, **5**:695-717, 1950.
233. VORHAUS, M. G.; GOMPERTZ, M. L.; and FEDER, A. Clinical experiments with riboflavin, inositol and calcium pantothenate, *Am. J. Digest. Dis.*, **10**:45-48, 1943.
234. WACHTEL, L. W.; HOVE, E.; ELVEHJEM, C. A.; and HART, E. B. Blood uric acid and liver uricase of zinc-deficient rats on various diets, *J. Biol. Chem.*, **138**:361-68, 1941.
235. WAISMAN, H. A. Production of riboflavin deficiency in monkey, *Proc. Soc. Exper. Biol. & Med.*, **55**:69-71, 1944.
236. WAISMAN, H. A., and MCCALL, K. B. A study of thiamine deficiency in the monkey (*Macaca mulatta*), *Arch. Biochem.*, **4**:265-79, 1944.
237. WAISMAN, H. A.; MCCALL, K. B.; and ELVEHJEM, C. A. Acute and chronic biotin deficiencies in the monkey (*Macaca mulatta*), *J. Nutrition*, **29**:1-11, 1945.
238. WALD, G. The photoreceptor function of the carotenoids and vitamins A. In: HARRIS, R. S., and THIMANN, K. V. (eds.), *Vitamins and hormones*, **1**:195-227. New York: Academic Press, Inc., 1943. See also The molecular basis of visual excitation, *Am. Scientist*, **42**:73-95, 1954.
239. WARBURG, O., and CHRISTIAN, W. Über ein neues Oxidationsferment und sein Absorptionsspektrum, *Biochem. Ztschr.*, **254**:438-58, 1932.
240. ———. Co-Fermentproblem, *ibid.*, **275**:464, 1935.
241. WHEELER, G. A.; GOLDBERGER, J.; and BLACKSTOCK, V. On probable identity of the Chittenden-Underhill pellagra-like syndrome in dogs and "black-tongue," *Pub. Health Rep.*, **37**:1063-69, 1922.
242. WHITE, E. A.; FOY, J. R.; and CERECEDO, L. R. Essential fatty acid deficiency in the mouse, *Proc. Soc. Exper. Biol. & Med.*, **54**:301-2, 1943.
243. WILLIAMS, R. D.; MASON, H. L.; SMITH, B. F.; and WILDER, R. M. Induced thiamine (vitamin B₁) deficiency and the thiamine requirement of man: further observations, *Arch. Int. Med.*, **69**:721-38, 1942.
244. WILLIAMS, R. J. Pantothenic acid—a vitamin, *Science*, **89**:486, 1939.
245. WILLIAMS, R. J., and MAJOR, R. T. The structure of pantothenic acid, *Science*, **91**:246, 1940.
246. WILLIAMS, R. J.; LYMAN, C. M.; GOOD-YEAR, G. H.; TRUESDAIL, J. H.; and HOLADAY, D. "Pantothenic acid," a growth determinant of universal biological occurrence, *J. Am. Chem. Soc.*, **55**:2912-27, 1933.
247. WINDAUS, A.; WESTPHAL, K.; WEIDER, F. V.; and RYGH, O. Einige Beobachtungen über die Ultraviolettbestrahlung

- der Ergosterins, Nachr. ü. d. Gesellsch. d. Wissensch. Göttingen, math.-phys. Kl., pp. 45-59, 1929.
248. WINTROBE, M. M.; BUSCHKE, W.; FOLLIS, R. H., JR.; and HUMPHREYS, S. Riboflavin deficiency in swine with special reference to occurrence of cataracts, Bull. Johns Hopkins Hosp., **75**:102-14, 1944.
249. WINTROBE, M. M.; FOLLIS, R. H., JR.; MILLER, M. H.; STEIN, H. J.; ALCAYAGA, R.; HUMPHREYS, S.; SUKSTA, A.; and CARTWRIGHT, G. E. Pyridoxine deficiency in swine with particular reference to anemia, epileptiform convulsions and fatty liver, Bull. Johns Hopkins Hosp., **72**:1-25, 1943.
250. WINTROBE, M. M.; FOLLIS, R. H., JR.; ALCAYAGA, R.; PAULSON, M.; and HUMPHREYS, S. Pantothenic acid deficiency in swine, Bull. Johns Hopkins Hosp., **73**:313-33, 1943.
251. WOLBACH, S. B., and BESSEY, O. A. Vitamin deficiency and the nervous system, Arch. Path., **32**:689-722, 1941.
252. ———. Tissue changes in vitamin deficiencies, Physiol. Rev., **22**:233-89, 1942.
253. WOLBACH, S. B., and HOWE, P. R. Tissue changes following deprivation of fat-soluble A vitamin, J. Exper. Med., **42**:753-77, 1925.
254. ———. Vitamin A deficiency in the guinea-pig, Arch. Path. & Lab. Med., **5**:239-53, 1928.
255. ———. The incisor teeth of albino rats and guinea pigs in vitamin deficiency and repair, Am. J. Path., **9**:275-94, 1933.
256. ———. Epithelial repair in recovery from vitamin A deficiency, J. Exper. Med., **57**:511-26, 1933.
257. WOOLLEY, D. W. A new dietary essential for the mouse, J. Biol. Chem., **136**:113-18, 1940.
258. ———. The occurrence of a "pellagra-genic" agent in corn, *ibid.*, **163**:773-74, 1946.
259. WOOLLEY, D. W., and KRAMPITZ, L. O. Production of a scurvy-like condition by feeding of a compound structurally related to ascorbic acid, J. Exper. Med., **78**:333-39, 1943.
260. WRIGHT, C. S. Vitamin D therapy in dermatology, Arch. Dermat. & Syph., **43**:145-54, 1941.
261. WRIGHT, C. S.; SAMITZ, M. H.; and BROWN, H. Vitamin B₆ (pyridoxine) in dermatology, Arch. Dermat. & Syph., **47**:651-53, 1943.
262. ZWEIFACH, B. W. The structural basis of permeability and other functions of blood capillaries, Cold Spring Harbor Symp. Quant. Biol., **8**:216-23, 1940.

CHAPTER 28

Pathophysiology of Blister Formation

I. SEPARATION AT THE EPIDERMAL-DERMAL JUNCTION	699
II. SEPARATION BETWEEN BASAL-CELL LAYER AND RETE	702
III. SEPARATION ABOVE THE BASAL LAYER (INTRAEPIDERMAL BLISTER FORMATION)	702

I. SEPARATION AT THE EPIDERMAL-DERMAL JUNCTION

MECHANICAL factors play a paramount role in the adherence of the epidermis to the corium. The elaborate system of interdigitations makes it difficult for the two layers to change their positions relative to each other. Two levels of interdigitations can be distinguished: (a) the papillae and interpapillary ridge system and (b) a fine digitation of basal cells which project slender processes into the connective-tissue ("root feet" or *Basalzellfüßchen*) (28) (Fig. 1). This junction can be likened to the so-called "mechanical joint," where the film of an adhesive substance becomes imbedded in the pores of the surfaces joined. Such a junction is more stable than if no pores are present (1, 11). Furthermore, there is histologic evidence that some reticular fibers of the dermis penetrate into the intercellular spaces of the epidermis (29, 39), thus adding an additional mechanical factor.

The adherence can be overcome by mechanical means. In human skin clear separation can be achieved by energetic suction (6) or by forceful stretching (45). In mouse skin, too, stretching facilitates separation (38). Flesch *et al.* (15, 14) achieved separation in laboratory animals after application to the skin of a group of unsaturated organic compounds.

The strength of the adherence at the junc-

tion is individually different. A genetically induced extreme degree of deficient adherence is exemplified in the dystrophic form of epidermolysis bullosa.

Whether, in addition to mechanical factors, there are other physicochemical forces holding the two layers together is not clear, because separation by apparently purely chemical means, such as peptic (43) and tryptic (24) digestion, by acids and by neutral salts (11), might be based primarily on differential swelling of the two layers; this again would mean mechanical displacement of papillae and interpapillary ridges from one another. This might be true also in the case of heat separation (2).

If one separates the epidermis from the dermis by any method, one is impressed by the stickiness of the dermal surface and finds it difficult to dismiss the role of this stickiness in the adherence. Since the discovery that an apparently homogeneous basement membrane exists on top of the dermis (17, 37) (p. 436) and that, according to histochemical evidence, this membrane consists mainly of mucopolysaccharides, one might think that the stickiness is due to the presence of these highly viscous compounds and that the stickiness must decrease (depolymerization?) when separation occurs. However, such a conclusion is not war-

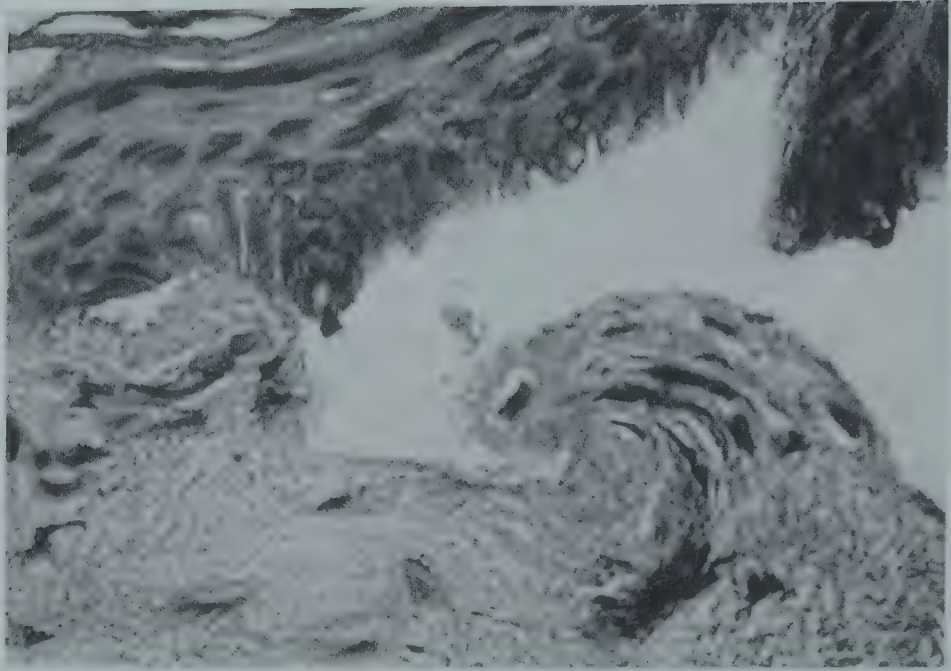


FIG. 1.— Section of human skin, showing the interdigitations at the junction of the epidermis and corium after separation. From Felsher (11). (Reproduced by permission of the Williams & Wilkins Company.)

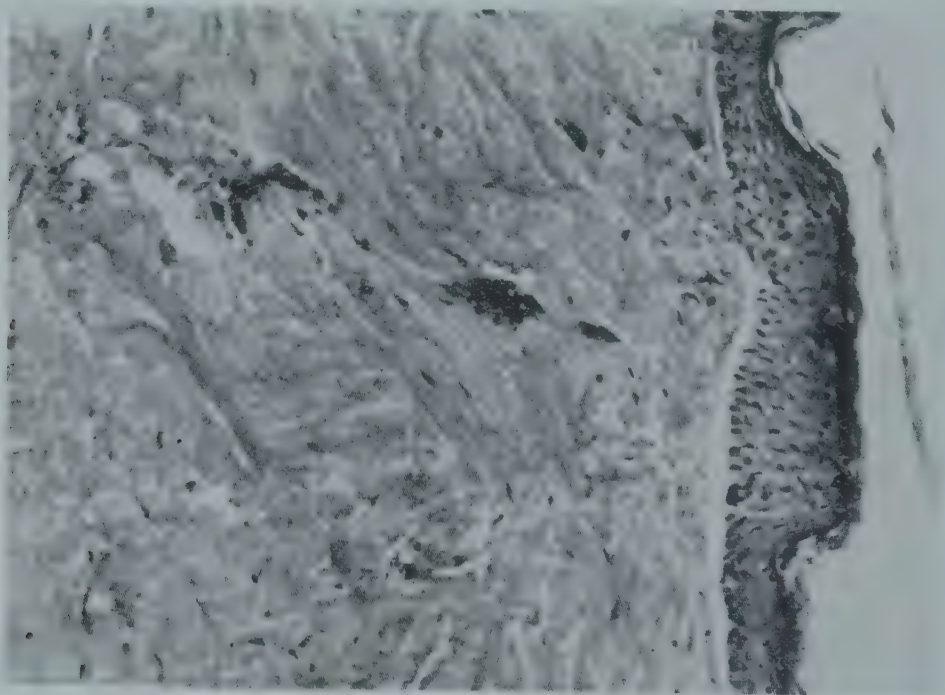


FIG. 2. Section of human skin which has been treated with a neutral salt solution, showing marked swelling of the collagenous fibrils after separation. From Felsher (11). (Reproduced by permission of the Williams & Wilkins Company.)

ranted, because (a) the basement membrane also contains nonmucopolysaccharide material, such as reticular and elastic fibers; (b) a similar stickiness is also present in deeper layers of the corium; and (c) collagen is known to have considerable cohesive and adhesive properties (11). What happens to the basement membrane when there is blister formation at the junction is hard to say, because it does not show any morphological changes, particularly no swelling. The apparently unchanged basement membrane remains on top of the dermis (36). Conversely, in many instances of junction separation the collagen fibers appear to be in a swollen state (Fig. 2).

Whether proteolytic enzymes, when separating the two layers, act by depolymerizing mucopolysaccharides or by proteolyzing collagen is not known. They can do both (17, 9).

In favor of the assumption that it is collagen which is altered or decomposed when separation is achieved by chemical means are the findings on separation with acids. Separation can be achieved at pH 3.4 (11). At this pH the swelling and hydrolysis of collagen is considerable (22), while mucopolysaccharides apparently swell very much less at this pH, because it is close to the isoelectric point of the ground substance, pH 3.8–4.2 (9). Separation by alkalies is also possible (2).

Separation by neutral salts (Fig. 2), as studied by Felsher (11), strongly indicates that swelling and subsequent peptization of collagen play a decisive role in breaking the adherence. Felsher demonstrated that anions of neutral salts are increasingly effective in the same order as they swell gelatin. The most effective anions are thiocyanates and iodides. In order of decreasing effectiveness, bromides > chlorides > acetates > sulfates > citrates follow. This order corresponds with the lyotropic series of Hofmeister, who found this order while experimenting on the swelling of gelatin (p. 405). The anions at the end of the series, particularly citrate, instead of facilitating separation by swelling, increase the adher-

ence because, instead of swelling, they cause shrinking mainly by dehydration (11).

The cationic series of Hofmeister is calcium > lithium > sodium > potassium in order of decreasing swelling capacity. The epidermis is separated in the same order in chloride solutions of these cations.

Still one cannot be sure whether these neutral salts act on collagen or on the ground substance or on both. According to histological findings of Kaye and Lloyd (18), neutral salt solutions cause swelling of both ground substance and collagen fibers.

The dermatological significance of Felsher's findings lies in their applicability to blister formation in dermatitis herpetiformis. In this blistering disease, all or most blisters form by separation at the epidermal-dermal junction (21). In a great number of patients with dermatitis herpetiformis, blisters can be provoked by local or systemic administration of iodides. Felsher (12) showed that thiocyanates are as effective or occasionally even more effective in this respect. Therefore, he assumed that in this disease the junction is pushed toward a loosened state through some swelling and/or peptization process by the unknown causative agent and that an additional push in this direction by thiocyanates or iodides will bring about complete separation. This interpretation explains why bromides, too, sometimes have a blister-provoking effect in this disease.

The significance of these findings is not diminished by claims that iodide sensitivity is not a constant phenomenon in dermatitis herpetiformis and that it is not specific for dermatitis herpetiformis, either, and therefore has no diagnostic value. All this might be true. Irregularities can be well explained by different degrees of the basic swelling state in different individual cases. Moreover, the testing method of applying potassium iodide in an ointment to the skin is not an ideal method, because of the variable penetration of iodides through intact skin; very little penetrates, if any (p. 36). Of course, a similar loosening process may take place occasionally in other blistering diseases. No

specificity of the loosening effect by a single causative agent is postulated.

Certainly, the nature of the iodide (and bromide) sensitivity, if and when it occurs in blistering skin diseases, has been com-

pletely clarified by Felsher's work (12), in which he demonstrated the equal effectiveness of thiocyanates. Earlier theories of a true iodide "allergy" or even of some thyroid disease(!) have become obsolete.

II. SEPARATION BETWEEN BASAL-CELL LAYER AND RETE

An observation of Felsher (11) proved that the cohesive forces between basal-cell layer and rete differ from those within the rete and from those at the epidermal-dermal junction. He treated excised human skin

tive to such treatment than is the adhesion of the rete to the basal cells.

Because of the morphologically fairly good state in which the basal cells were left behind, one may also assume that the ma-



FIG. 3.—Section of human skin treated with half-saturated calcium hydroxide solution at pH 12, showing the basal cells still adhering to the corium. From Felsher (11). (Reproduced by permission of the Williams & Wilkins Company.)

with half-saturated calcium hydroxide solution at pH 12 and found that the basal cells remained adherent to the dermis, while the rest of the epidermis was separated (Fig. 3). Thereby he demonstrated that the adhesion between epidermis and corium is less sensi-

terial connecting the basal layer to the rete is more sensitive to treatment with lime water than are the basal cells themselves. The reason why the basal layer remains adherent while the rete is washed off remains to be investigated.

III. SEPARATION ABOVE THE BASAL LAYER (INTRAEPIDERMAL BLISTER FORMATION)

If there is damage to the superficial minute vessels of the skin, with leakage of plasma or serum through the vessel walls, the fluid enters intracellular spaces in the

epidermis and may separate cell layers or single cells from one another simply by pressure. Blister formation in physical and chemical burns could have such a mecha-

nism, although primary chemical effects on the epidermis have never been excluded. On the contrary, evidence is accumulating that primary epidermal damage contributes to the disintegration of epidermal cells when blisters are formed. The fact that urticarial wheals and inflammatory papules in most cases do not blister, in spite of the accumulation of large amounts of fluid in the papillary layer, suggests that epidermal blisters can hardly be produced merely by hydrodynamic pressure of accumulated fluid, as was assumed by Percival and Hannay (30).

To explain intraepidermal blister formation by pressure from inflammatory exudates is most difficult in the case of so-called "spongiotic blistering" as it occurs in the eczematous type of dermatitis. In spongiosis there is, first, a widening of the intercellular canals in the rete, apparently by fluid accumulation; the intercellular bridges are stretched and finally broken. Thereby the cells become separated from one another, and, as this disintegration progresses, first microscopic and later macroscopic cavities form which are filled with fluid. Morphologically, the process looks as if it were based on fluid accumulation and pressure. But it is improbable that the fluid stems from damaged blood vessels, because, first, spongiotic vesicles often appear first in the upper half of the rete without any derangement of the lower half, and, second, spongiotic vesiculation by no means parallels the vascular damage. A remarkable observation was made lately by Bizzozero (5), who found that more intense spongiosis can be produced by croton oil if it is applied from the outside than if injected intradermally, although the latter form of application is likely to cause considerably more vascular damage.

Altogether, spongiotic reaction is known to occur more frequently following the application of irritative substances from the outside than under any other condition. If fluid accumulates, it probably comes from damaged epidermal cells. If the cell membrane is damaged, it may become abnormally permeable to salts; and if the

electrolyte concentration of intercellular spaces increases, osmotic forces will drive water from the cells into these spaces.

While this is pure speculation, it seems reasonable to assume at least that spongiotic vesiculation has a mechanism quite different from other types of blistering, first, because of its relatively great independence of vascular damage and, second, because of the behavior of the intercellular bridges. Microscopic observations indicate that these bridges resist stretching for a long time, and even after they are broken they may persist without being dissolved. Thus spongiotic blister formation seems not to be connected with any selective attack on the fibrillary elements in the epidermis (cf. p. 344).

In contrast, blister formation in pemphigus vulgaris and possibly in other bullous dermatoses sets in, according to histological findings, with dissolution of the intercellular spines, or "acantholysis." No matter whether this acantholysis can be observed consistently in certain diseases and not in others, it is highly significant that in some instances acantholysis does occur beyond doubt. It indicates a selective action of the noxious agent on the fibrillary material in the epidermis.

Thus there are at least two different and distinguishable mechanisms in intraepidermal blister formation: primary widening of intercellular spaces, with preservation of spines, and acantholysis, or primary dissolution of spines.

If there is chemical damage to the epidermis introducing blister formation, what is its nature in particular instances? The first systematic study was done by Peters, Thompson, and their associates (41) on the mechanism of blister formation by the arsenical vesicant, lewisite. They demonstrated that lewisite acts by binding the sulfhydryl groups of cellular proteins with enzymatic functions (p. 571). By the inhibition of these sulfhydryl groups, the vitamin B₁-containing enzyme, pyruvic acid dehydrogenase, becomes unable to oxidize pyruvic acid, and the metabolism of the cell is profoundly disturbed (41).

The correct recognition of this mechanism led to the elaboration of the British antilewisite BAL (dimercaptopropanol or "dimercaprol") (31). By virtue of two sulfhydryl groups on adjacent carbon atoms, dimercaprol is able to prevent the binding of sulfhydryl groups in tissue proteins by arsenicals and by heavy metals. Or, if such binding has already occurred, dimercaprol releases the cellular sulfhydryl groups by forming a stable 5-membered ring with arsenic and similar compounds with heavy metals (34). This stable ring compound is excreted in the urine. This is the basis of the detoxicating effect of BAL (34, 42).

Similar to arsenicals, iodoacetic acid and its amide are poisons with great affinity for free sulfhydryl groups. They are blistering agents (44, 23), and their effect is based on their sulfhydryl-binding capacity (13).

Another instance of enzymatic inhibition leading to blister formation was demonstrated by Dixon and Needham (10). These authors used chemicals inhibiting the enzyme hexokinase which phosphorylates glucose to glucose-6-phosphate. Most effective among such compounds is methyl bromide. In the absence of hexokinase, the tissue is unable to utilize glucose. All chemicals inhibiting hexokinase cause blistering if applied to the skin.

While in these instances exogenous toxic substances cause the damage, evidence is accumulating that endogenous material may also be responsible for blister formation. This endogenous material apparently is liberated in the epidermis following injury.

The presence of proteases and peptidases in skin extracts was first demonstrated thirty-five years ago by Sexmith and Petersen (33). In modern times Peters and his associates initiated a thorough study of the proteolytic enzymes of the skin (3, 4, 27). Important contributions were also made by Fruton (16).

The result of these investigations so far has been that there are at least three well-characterized proteolytic enzymes present in the skin: (1) a "dermoprotease," which, although an endopeptidase like trypsin and

chymotrypsin, is clearly different from these enzymes by its failure to digest typical synthetic substrates for trypsin and chymotrypsin; (2) a peptidase (leucine aminopeptidase), which splits leucine amide and is similar to peptidases isolated from other tissues; and (3) a dermopeptidase different in its specificity from all known enzymes.

A further important step in the study of proteolytic enzymes was made by G. C. Wells (46). He separated epidermis and corium of human skin from fresh surgical specimens by the stretch method of Van Scott (45) and demonstrated that the great bulk of the protease is located in the epidermis (Fig. 4). This protease he called the "epidermo-protease." Its specific features are now being investigated.

There is reason to believe that damage to the epidermal cells may lead to the liberation of the enzymes from within the cells. This is borne out by findings of Zamecnik *et al.* (47), who recovered one of the skin-specific peptidases from blister fluid in burns. Another impressive piece of evidence was presented by Cullumbine and Rydon (7). These authors studied Menkin's leukotaxine (25), a polypeptide found in inflammatory exudates, which causes increase in capillary permeability and rapid migration of leukocytes through the capillary wall. This substance is supposed to be produced in tissue injury and to be the cause of the acute inflammatory reaction (p. 100). Cullumbine and Rydon, using Peters' dermoprotease, found that if this enzyme acts on blood fibrin, the split products yield a substantial amount of leukotaxine activity. Thus dermoprotease may be responsible for the production of leukotaxine in the early stages of vesiculation.

There are two possible mechanisms whereby the proteolytic enzymes of the skin may become active after injury. One is liberation from the cell by direct action of the injurious stimulus on the cell periphery. The other is destruction or inactivation of some inhibitor of the enzyme by the injury, so that the normally inactive enzyme becomes activated. The latter possibility was

thought of, because intradermal injection of dermoprotease preparations in themselves do not cause rapid liberation of leukotaxine (7).

In the field of acantholytic blister formation the recent findings of Stoughton (35) are significant. He succeeded in isolating from feces of patients with ulcerative colitis

which selectively attacks the spines. Interestingly, Stoughton found that sulfated compounds, such as suramin sodium and treburon, which have shown promise in the symptomatic treatment of pemphigus vulgaris, inhibit the activity of factor A.

Acantholysis was observed also in experimental burns (20). It seems that the spines

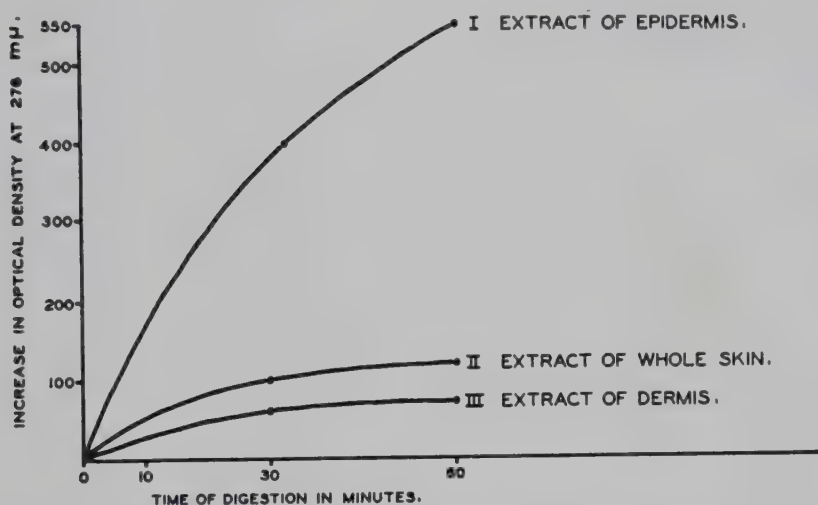


FIG. 4.—Proteolytic activity of epidermis and dermis. Hemoglobin substrate. From Wells (46). (Reproduced by permission of the Williams & Wilkins Company.)

an enzyme which has two distinctly different actions: a factor B, which is similar to trypsin in all respects, and a factor A, which has unique properties. When incubated with formalin-fixed sections of human skin, this factor produces a histological picture reminiscent of pemphigus vulgaris, with primary acantholysis and secondary blister formation. Thereby it became feasible to postulate in pemphigus the presence of an enzyme

play an important role in the maintenance of the integrity of the epidermis. Therefore, the chemical nature of these spines and their specific digestion by enzymatic factors deserves further investigation.

Lately, emphasis has been laid upon the role of calcium in maintaining the coherence of rete cells (p. 503). Szodoray *et al.* (40) found a conspicuously low calcium content in the epidermis of pemphigus blisters.

BIBLIOGRAPHY

- ALEXANDER, J. Colloid chemistry: principles and application. New York: D. Van Nostrand Co., Inc., 1937.
- BAUMBERGER, J. P.; SUNTZEFF, V.; and COWDRY, E. V. Methods for separation of epidermis from dermis and some physiologic and chemical properties of isolated epidermis, *J. Nat. Cancer Inst.*, **2**:413-23, 1942.
- BELOFF, A. Skin protease inhibitory factor of plasma, *Biochem. J.*, **40**:108-15, 1946.
- BELOFF, A., and PETERS, R. A. Observations upon thermal burns; influence of moderate temperature burns upon proteinase of skin, *J. Physiol.*, **103**:461-76, 1945.
- BIZZOZERO, E. Dermatiti fossiche e spongiosi, *Dermatologica (suppl.)*, **97**:7-10, 1948.
- BLANK, I. H., and MILLER, O. G. A method for the separation of epidermis from dermis, *J. Invest. Dermat.*, **15**:9-10, 1950.
- CULLUMBINE, H., and RYDON, H. N. A

- study of the formation, properties and partial purification of leukotaxine, *Brit. J. Exper. Path.*, **27**:33-46, 1946.
8. DAY, T. D. Influence of hydrogen ions and natural salts upon the hydration of interstitial connective tissue, *Nature*, **162**:152-53, 1948.
 9. ———. The permeability of interstitial connective tissue and the nature of the interfibrillary substance, *J. Physiol.*, **117**:1-8, 1952.
 10. DIXON, M., and NEEDHAM, D. M. Biochemical research on chemical warfare agents, *Nature*, **158**:432-38, 1946.
 11. FELSHER, Z. Studies on the adherence of the epidermis to the corium, *Proc. Soc. Exper. Biol. & Med.*, **62**:213-15, 1946; also *J. Invest. Dermat.*, **8**:35-47, 1947.
 12. ———. The nature of halogen sensitivity in dermatitis herpetiformis and pemphigus, *ibid.*, pp. 55-58.
 13. FLESCH, P., and GOLDSTONE, S. B. Irritant action of iodoacetic acid, *J.A.M.A.*, **144**:1110, 1950.
 14. FLESCH, P.; GOLDSTONE, S. B.; and WEIDMAN, F. D. Blister formation and separation of the epidermis from the corium in laboratory animals, *J. Invest. Dermat.*, **18**:187-92, 1952.
 15. FLESCH, P.; KLIGMAN, A. M.; and BALDRIDGE, G. D. An improved method for the separation of the epidermis of laboratory animals, *J. Invest. Dermat.*, **16**:81-84, 1951.
 16. FRUTON, J. S. On the proteolytic enzymes of animal tissue. V. Peptidases of skin, lung and serum, *J. Biol. Chem.*, **166**:721-38, 1946.
 17. GERSH, I., and CATCHPOLE, H. R. The organization of ground substance and basement membrane and its significance in tissue injury, disease and growth, *Am. J. Anat.*, **85**:457-521, 1949.
 18. KAYE, M., and LLOYD, D. J. A histological and physico-chemical investigation of the swelling of a fibrous tissue, *Proc. Roy. Soc. London, s.B.*, **96**:293-316, 1924.
 19. KING, L. S. Effects of podophyllin on mouse skin. III. A study of epidermal fibrils, *J. Nat. Cancer Inst.*, **10**:689-709, 1950.
 20. LEACH, E. A.; PETERS, R. A.; and ROSSITER, R. S. Experimental thermal burns, especially moderate temperature burn, *Quart. J. Exper. Physiol.*, **32**:67-86, 1943-44.
 21. LEVER, W. F. *Histopathology of the skin*. Philadelphia: J. B. Lippincott Co., 1949.
 22. LLOYD, D. J., and SHORE, A. *Chemistry of the proteins*. London: J. & A. Churchill, 1938.
 23. MARCUS, M. D., and FRERICHs, J. B. Dermatitis due to contact with iodoacetic acid, *J.A.M.A.*, **142**:805-6, 1950.
 24. MEDAWAR, P. B. Sheets of pure epidermal epithelium from human skin, *Nature*, **148**:783, 1941.
 25. MENKIN, V. *Dynamics of inflammation*. New York: Macmillan Co., 1940.
 26. NELEMANS, T. G.; KEUNING, F. J.; RYSEL, T. G. VAN; and RUITER, M. Histological changes in the tonofibrils in vesicular and bullous diseases of the skin, *Brit. J. Dermat.*, **64**:177-89, 1952.
 27. NEVILLE-JONES, D., and PETERS, R. A. Further observations on the proteolytic enzymes in rat skin, *Biochem. J.*, **43**:303-8, 1948.
 28. ODLAND, G. F. The morphology of the attachment between the dermis and the epidermis, *Anat. Rec.*, **108**:399-413, 1950.
 29. PAUTRIER, L. M., and WORINGER, F. Contribution à l'étude de l'histophysiologie cutanée; les rapports morphologiques entre l'épiderme et le derme, *Ann. de dermat. et syph.*, **1**:985-1005, 1930.
 30. PERCIVAL, G. H., and HANNAY, P. W. Observations on the structure and formation of bullae. I and II, *Brit. J. Dermat.*, **61**:41-54 and 77-89, 1949.
 31. PETERS, R. A.; STOCKEN, L. A.; and THOMPSON, R. H. S. British anti-lewisite (BAL), *Nature*, **156**:616-19, 1945.
 32. ROTHMAN, S., and SCHAAF, F. *Chemie der Haut*. In: JADASSOHN, Handb. d. Haut- u. Geschlechtskr., **1/2**:161-377. Berlin: J. Springer, 1929.
 33. SEXMITH, E., and PETERSEN, W. F. Skin ferments, *J. Exper. Med.*, **27**:273, 1917. Quoted by ROTHMAN and SCHAAF (32).
 34. STOCKEN, L. A., and THOMPSON, R. H. S. British anti-lewisite. I. Arsenic derivatives of thiol proteins, *Biochem. J.*, **40**:529-35, 1946.
 35. STOUGHTON, R. B. Enzymatic cytolysis of epithelium by filtrates of feces from patients with ulcerative colitis, *Science*, **116**:37-39, 1952.
 36. ———. Personal communication.
 37. STOUGHTON, R. B., and LORINCZ, A. L. The

- action of collagenase on skin and the anti-collagenase factor in human serum, *J. Invest. Dermat.*, **16**:43-52, 1951.
38. SUNTZEFF, V., and CARRUTHERS, C. The water content in the epidermis of mice undergoing carcinogenesis by methylcholanthrene, *Cancer Research*, **6**:574-77, 1946.
39. SZODORAY, L. The structure of the junction of the epidermis and the corium, *Arch. Dermat. & Syph.*, **23**:920-25, 1931.
40. SZODORAY, L.; VÉRTES, F.; RÁCZ, S.; and HORVÁTH, G. Über einige aktuelle Fragen der Pathogenese des Pemphigus, *Dermatologica*, **103**:125-35, 1951.
41. THOMPSON, R. H. S. The effect of arsenical vesicants on the respiration of the skin, *Biochem. J.*, **40**:525-29, 1946.
42. ———. Enzymic mechanisms in skin, *Tr. St. John's Hosp. Dermat. Soc.*, No. 31, pp. 1-6, 1952.
43. UNNA, P. G. *Biochemie der Haut*. Jena: G. Fischer, 1913.
44. VÁMOS, L. VON; VÉRTES, F.; and RÁCZ, S. Zum Mechanismus der Blasenbildung, *Dermatologica*, **99**:378-84, 1949.
45. VAN SCOTT, E. J. Mechanical separation of the epidermis from the corium, *J. Invest. Dermat.*, **18**:377-79, 1952.
46. WELLS, G. C., and BABCOCK, C. Epidermal protease, *J. Invest. Dermat.*, **21**:459-63, 1953.
47. ZAMECNIK, P. C.; STEPHENSON, M. L.; and COPE, O. Peptidase activity of lymph and serum after burns, *J. Biol. Chem.*, **158**:135-44, 1945.

Subject Index

- Absorption: barrier of, 28, 29, 30, 32, 33, 35, 36, 37, 46, 47, 50; effect of blood flow, 32-33; effect of concentration, 81-82; effect of electrophoresis, 50-51; effect of essential oils, 49; effect of horny layer, 31-32, 240; effect of keratolytics, 32; effect of lipid surface film, 31; effect of moisture, 52; effect of oil-in-water emulsions, 48-49; effect of organic solvents, 49; effect of stimulation, 49-50; effect of vehicles, 47-49; methods of estimation, 34; percutaneous, 26-59; of single compounds, 35-45; transepidermal, 35, 47; transepidermal, lipid theory, 29; transepidermal, membrane theory, 27, 28; transfollicular, 29, 33, 47, 49, 52; transudoriferous, 33, 34
- Acantholysis, 703, 705
- Acanthosis nigricans, 550
- Acclimatization to heat, 158, 161, 190, 211, 213, 215, 265, 266
- Acetaldehyde, 472
- Acetic acid (incl. acetate), 567, 570, 576, 701
- Acetoacetic acid, 664
- Acetyl; *see under* parent compound
- Acetylcholine, 47, 81, 90, 91, 94, 101, 143, 160-66, 168, 169, 173-75, 179, 183, 549, 564, 570, 576, 582, 672, 676; muscarinic action of, 91, 169-71; nicotinic action of, 168-73
- Achard-Thiers syndrome, 630
- Achromotrichia (incl. graying of hair), 532, 541, 542, 543, 666, 672, 674, 676, 688; anti-achromotrichial vitamin, 543
- Acid mantle, 221
- Acid neutralization, 227, 229
- Acid-base balance, 202, 211, 222, 281
- Acne, 188, 211, 298, 299, 300, 385, 575, 680
- Aconitase, 476, 568
- Acroasphyxia, 67
- Acrodermatitis atrophicans, 3, 509
- Acrodynia, 670, 685
- Acromegaly, 499, 550
- Action currents: of sensory impulses, 126-28; of sweat impulses, 19, 20
- Adaptation, sensory, 126, 129, 130, 176
- Addison's disease; *see* Adrenal insufficiency
- Adenine, 668
- Adenosine diphosphate, 570
- Adenosine triphosphate, 373, 566, 567, 571
- Adenylpyrophosphatase, 575
- Adrenal glands: and hair growth, 41, 625-26, 629-30; medulla of, 546, 549; and pigmentation, 546-47; vitamin C content of, 677
- Adrenal insufficiency (incl. Addison's disease), 108, 214, 215, 266, 534, 544, 550, 626, 628, 629
- Adrenal rest tumors, 301, 627
- Adrenaline; *see* Epinephrine
- Adrenergic impulses, 85, 86, 88, 161, 166-68, 183-84
- Adrenochrome, 526
- Adrenocortical hormones, 103-4, 499; *see also under* single hormones
- Adrenocorticotrophic hormone (ACTH), 103, 145, 265, 299, 300, 439, 441, 442, 444, 453, 509, 544, 545, 625, 640
- Afterdischarge, sensory, 129, 130
- Age differences: in apocrine glands, 182, 183; in calcium content, 503; in capacitance reactance, 15; in carbon dioxide delivery, 582; in cholesterol content, 485; in cholesterol excretion, 325, 328; in fat content, 483; in globular proteins, 347; in glycolysis, 472, 571; in hair properties, 619-21; in lipase activity, 576; in phospholipid content, 488; in resiliency, 3; in sebum excretion, 295-97; in squalene excretion, 328; in sulfur content, 341-42; in sweat pH, 223; in tensile strength, 5; in tension, 1; in water content, 495
- Agerite alba, 534
- Agnosterol, 326
- Alanine, 207, 208, 338, 347, 396, 403, 412, 634; β -, 347
- Albino, 550
- Albright's syndrome, 550
- Albuminoids, 340
- Alcohol intake, 165, 233, 254
- Alcohols, 309, 312, 313, 314, 321-27, 331; dihydroxy-, 321
- Aldobionic acid, 431
- Aldolase, 567
- Aldrich-McClure test, 27, 419
- Alimentary toxicosis, 4
- Alkali neutralization, 227-30; sensitivity, 227
- Alkaloids, 38, 42, 46
- Alkalosis, 222, 236, 281
- Alkaptonuria, 664, 665
- Alkyl-phosphate, 101
- Allergens; *see* Antigens
- Alopecia (incl. hair loss), 622, 624, 631-40, 641, 644; areata, 611, 622, 623, 624, 640; due to chemicals, 632-34; due to ionizing radiation, 634-39; due to nutritional deficiencies, 664, 666, 667, 673, 675, 676, 685; frontal, 627, 630-32; peroneal, 632; physiological, 631-32; postinfectious, 623, 639-40; *see also* Baldness; Calvities
- Aluminum, 11, 277, 642, 685, 686
- Amides, 339, 354
- Amino acid antagonists, 633, 634
- Amino acids, 202, 204, 207-9, 224, 228, 337-41, 347-48, 351, 354, 368, 371, 372, 374, 392, 395, 409, 412, 434, 447, 499, 517, 541, 542, 567, 571, 573, 621, 663-66; acid and base binding by, 340; deficiency in, 663-65; diamino, 222, 339, 353, 357; dicarboxylic, 222, 339, 353, 354, 357; dipolar nature of, 339; essential, 351, 368, 623, 624; periodicity of, 340, 354, 399, 400
- Aminopterin, 677
- Amitosis, 589
- Ammonia (incl. ammonium salts), 186, 208-9, 347, 348, 374, 530, 574
- Amyl nitrite, 233
- Amyloidosis, 451, 597; par-, 451
- Anaphylactic shock, 97
- Anemias, 550; pernicious; *see* Pernicious anemia
- Anidrosis, 165, 175; thermogenic, 270
- Anilino-quinone, 523, 534
- Anteisoacids, 315, 316, 321, 322
- Anteisoalcohols, 321
- Anti-alpecia factor, 622
- Anticholinesterases, 163, 169
- Anticollagenase, 513
- Antidromic vasodilatation, 89-91, 94, 140, 175, 188
- Antigens (incl. allergens), 34, 42, 44, 51, 94, 272, 394, 441, 452, 539

- Antihistaminic drugs, 42, 99, 105, 142, 180, 572
 Antihyaluronidase, 572
 Antimony, 43, 645
 Antinicotinic drugs, 91, 173
 Antipyrine, 33
 Antisodium barrier, 508
 Apocrine glands, 168, 177, 180–88, 216, 223, 236, 310; age differences in, 182, 183; histochemistry of, 190–92; innervation of, 183; racial differences in, 180; sex differences in, 182, 183; stimulation of, 183; structure of, 154–55; *see also* Sweat glands
 Apocrine sweat: composition of, 186–88; pH of, 221, 222; urea content of, 204; *see also* Sweat
 Aqueous surface film: composition of, 201–20; pH of, 221–32; tonicity of, 235
 Arachidic acid, 314
 Arachidonic acid, 684, 685
 Arginase, 204, 348, 574
 Arginine, 204, 339, 340, 343, 344, 347, 348, 352, 353, 372, 396, 411, 412, 574
 Arginine cycle of Krebs, 207, 208
 Argyrophil fibers, 409–10, 443; *see also* Reticulin
 Arrhenoblastoma, 626, 627
 Arsenicals, 43, 434, 545, 550, 570, 632, 640–41, 685
 Arterenol, 530
 Arteriovenous anastomoses, 61, 75, 77–79, 87, 104, 256, 262
 Aryl-phosphates, 101
 • Ascorbic acid, 41, 212, 433, 443, 444, 534, 543, 546, 550, 665, 677, 679, 681; dehydro-, 677; gluco-, 677
 Asparagin, 207
 Aspartic acid, 207, 222, 339, 340, 347, 354, 396, 411, 412
 Asteatosis, 224, 225, 291, 294, 357, 378–80, 495, 496
 Atabrine, 272, 632, 666
 Atheromata, 324
 Athlete's foot; *see* Fungus infections
 Atomic bomb explosions, 639
 Atopic dermatitis (incl. atopy, atopic eczema, atopic individuals, flexural dermatitis, infantile eczema, neurodermatitis exudative and dry), 17, 80, 91, 138, 142, 225, 227, 230, 294, 510, 618, 675, 685, 688; vasoconstriction in, 80, 81, 94; *see also* "Eczema"
 ATP-phosphoglyceric transphosphorylase, 567
 ATP-phosphopyruvate transphosphorylase, 567
 Atropine (incl. atropinization), 13, 18, 19, 21, 46, 91, 156, 162, 163, 166, 169, 172, 184, 213, 228, 233, 236, 238
 Avidin, 673
 Axon reflex: hyperalgesic, 133; pilomotor, 94, 170–71, 173; sweating, 94, 166, 168–73, 176; vasodilatory (flare), 91–94, 170
 Baldness, 631; common male, 630, 632; *see also* Alopecia
 Banthine, 101, 163
 Barium, 36, 541
 Barrier of absorption, 28, 29, 30, 32, 33, 35, 37, 46, 47, 50
 Basal metabolic rate, 238
Basalzellfüßchen, 699
 Basement membranes, 30, 433, 436, 437, 439, 447, 450, 452, 699, 701
 Beard, 629–30
 Beeswax, 321
 Behenic acid, 314
 Benzene, 280
 Beriheri, 665, 688
 Beryllium, 576
 Besnier's prurigo, 225
 Bier spots, 71
 Biliverdin, 677
 Biotin, 211, 371, 543, 623, 673–75
 Birefringence, 367, 374, 403–4, 412
 Bismuth, 43, 645
 Bizzozero nodules, 367
 Blacktongue, 668, 669
 Blisters (incl. blistering, bullae, vesicles), 99, 226, 239, 511, 576, 668, 699–709
 Blood flow: and absorption, 32–33; direct observation of, 64–69; in inflammations, 233; in irritated skin, 612, 619; rate of, 70, 74–76, 257, 261, 581, 631; and sensory thresholds, 135, 136; and sweat secretion, 188–89
 Blood pressure, capillary, 72–74, 83, 233, 257
 Blushing reaction, 75, 91, 189
 Boron, 642, 685, 686
Boule d'œdème, 419
 Boundary between cornified and noncornified epidermis, 13, 16, 29, 32, 33
 Bridges, intercellular (incl. spines), 344, 345, 586, 703, 705
 British antilewisite (BAL), 704
 Bromides, 51, 642, 701, 702
 Bromidrosis, 188
 Bromobenzene, 644–45
 Buerger's disease, 66
 Buffering capacity, 211, 227–30
 Butyric acid, 222, 316
 Calciferol, 681, 682
 Calcinosis, 510
 Calcium, 11, 36, 64, 130, 143, 172, 202, 215, 216, 376, 452, 476, 502–6, 509–10, 541, 576, 641, 642, 685, 686, 702, 705
 Calluses, 31, 251, 377, 378, 379
 Calvities, 631; frontalis adolescentium, 631
 Camphor, 40
 Candida albicans, 478
 Cannizaro reaction, 322
 Cannon's rule, 163
 Capacitance reactance, 14, 15
 Capillaries: blood pressure in, 72–74, 257; contractility of, 76–77; of nail fold, 64–65, 68–79; periodic contraction of, 67, 75, 85, 100; permeability of, 64, 442, 686, 704
 Capillary microscopy, 64–69, 102, 104
 Capric acid, 314
 Caproic acid, 316
 Capsaicine, 176
 Carbohydrates, 186, 396–97, 409, 422, 425, 432, 465–82, 565–68, 571, 623; *see also* under single compounds; Mucopolysaccharides; Polysaccharides
 Carbon dioxide: absorption of, 32, 45; and buffering, 228–29; delivery to outside, 580–83; and vasodilatation, 101
 Carbon tetrachloride, 280
 Carbonic anhydrase, 688
 Carboxylase, 472, 686
 Carnaubyl alcohol, 321
 Carotenase, 680
 Carotenes, 41, 330, 516, 518, 679, 680
 Catalase, 575
 Cataphoresis, 51
 Catechol, 521, 523, 530
 Causalgia, 134
 Cell conversion, 590
 Cement substance, intercellular, 64, 407, 452, 684
 Cerotic acid, 314, 315
 Cerumen, 288, 328
 Ceryl alcohol, 321
 Ceryl palmitate, 321
 Cesium, 36
 Cetyl alcohol, 321
 Chapping, 378–80
 Cheilosis, 666, 670
 Chelating agents, 542
 Chicken pox, 99
 Chilblain, 262
 Chitin, 422
 Chitosamine, 422
 Chloasma, 547, 550
 Chlorides, 13, 14, 35–37, 190, 202, 211, 212–15, 222, 235, 265–66, 281, 498, 500–501, 508–9, 530–31, 533, 623, 685, 701
 Chlorine gas, 30
 Chloroform, 277, 280
 Chloroprene dimers, 630, 633, 638
 Δ^7 -Cholestenol, 323, 487
 Cholesterol, 31, 37, 186, 191, 309, 312, 323–25, 327, 328, 329, 331, 485–89, 577, 611, 623, 624; 7-dehydro-, 323, 329, 681; dihydro-, 323, 324, 485; esteri-

- fication of, 325, 486; esters, 31, 322, 331, 486-87; ester/total, 327; 7-hydroxy-, 324, 330; 7-keto-, 323; meta-, 323; oxy-, 323
 Cholesterol palmitate, 486
 Choline, 209, 488, 489-90, 543, 570, 576, 676; esters, 169
 Cholinergic impulses, 88, 90, 91, 94, 162-65, 179, 183-84
 Cholinesterases, 90, 97, 101, 161, 184, 185, 186, 331, 564, 570, 576
 Chondriogenes, 540
 Chondroitin sulfuric acid (incl. sulfate), 341, 396, 421-25, 432, 434, 435, 437, 443, 446, 449, 452-53; sulfate B, 432
 Chondromucinate, 432
 Chondrosamine, 396, 422, 424
 Chromatophores, 520, 544, 549
 Chromidrosis, 186
 Chromium, 530, 642
 Chromodacryorrhea, 673
 Chromoprotein, 340
 Chymotrypsin, 573
 Circulation, 60-119
 Cis-aconitic acid, 473, 474
 Citramide, 374
 Citric acid (incl. citrate), 374, 473, 474, 476, 567, 568, 701
 Citrulline, 207, 208, 347
 Clear cells, 536
 Cleavage lines, 3
 Clo, 260
 Clothing, 247, 259-60, 603, 604
 Club hairs, 606-9, 638
 Cobalt, 530, 642, 685
 Cobefrin, 530
 Cocaine, 176
 Cocarboxylase, 472, 477, 665
 Coenzymes, 565, 566, 568, 571; A, 665, 672; I, 668; II, 668
 Colchicine, 595
 Cold, local effects of, 260-64
 Cold creams, 48; isotopic, 44
 Cold-handed persons, 78
 Cold spots, 121, 122, 123, 146
 Cold-waving process, 356
 Collagen (incl. collagen fibers, fibrils, etc.), 312, 341, 359, 411, 420, 421, 422, 425, 437, 438, 444, 446, 447, 451, 453, 495, 499, 566, 571, 572, 573, 577, 700, 701; action of enzymes on, 397; amino acid content of, 395-96; birefringence of, 403-4; carbohydrate content of, 396-97; cross-striation of, 401-3, 407, 408, 450; effect of hormones on, 398-99; electron microscopy of, 407-9; general properties of, 393-94; isoelectric point of, 395, 407; molecular weight of, 396, 397; nitrogen content of, 341, 394; purification of, 394-95; shrinkage of, 405; swelling of, 405-7
 "Collagen diseases," 409, 451, 452
 Collagenase, 397, 433, 437, 573
 Colloid osmotic pressure, 72, 73, 663
 Color of skin, 69-72, 80, 253, 257, 516-18
 Comedones, 300
 Congelation, 262
 Connective-tissue repair, 442-46
 Copper, 43, 371, 372, 375, 528, 530, 531, 532, 540, 541, 542, 543, 545, 565, 568, 623, 641, 642, 643, 672, 677, 685, 686, 688
 Coproporphyrin, 673
 Coramine, 668
 Corio-mucoid, 423
 Corns, 31, 378
 Cortin, 103
 Cortisone, 41, 145, 299, 300, 398, 399, 433, 441, 442, 444-46, 451, 453, 509, 544, 547, 572, 629, 640, 641
 Creatine, 205
 Creatinine, 205-6
p-Cresol, 521
 Cushing's syndrome, 3, 7, 300, 550, 625, 629
 Cutis laxa, 6, 450
 Cutis marmorata, 70, 71
 Cutis verticis gyrata, 3
 Cyanocobalamine, 677
 Cysteic acid, 347
 Cysteine, 338, 340, 341, 368, 546, 644, 645; homo-, 369
 Cystine, 338, 341, 342, 343, 344, 347, 348, 351, 352, 353, 356, 357, 368, 369, 372, 387, 395, 396, 412, 542, 543, 621, 623, 633, 644, 664
 Cystinuria, 664
 Cytochrome: a, 568; b, 568; c, 371, 530, 568, 569; oxidase, 371, 540, 569, 571; pigments, 568; reductases, 568; systems, 567, 568, 571, 665
 Cytocrine theory, 538, 540

 Darier's disease, 385
 Dark adaptation, 680
 DDT, 46
 Deamination, oxidative, 348
 Depigmentation, 532; postinflammatory, 535
 Depilatories, 356
 Depolarization, 11, 13, 14, 21
 Depot function of skin: for calcium, 503; for electrolytes, 496-99; for fats, 484; for water, 483, 496
 Dermatitis (incl. inflammation, inflammatory processes), 14, 20, 130, 254, 258, 261, 272, 508, 550, 582, 583, 703; contact, 141, 142, 227, 279, 508, 602; eczematous type, 99, 226; exfoliative, 226, 234, 261, 324, 353, 377, 486, 489, 508, 632; herpetiformis, 550, 669, 701; seborrheic, 80, 160, 211, 221, 224, 229, 272, 294, 508, 644, 666, 670, 674; *see also* Atopic dermatitis; "Eczema"
 Dermatomes: segmental, 132; sympathetic, 178
 Dermatomyositis, 394, 450, 573
 Dermographia alba, 79, 84
 Dermographia elevata, 84
 Dermographia rubra, 82, 84
 Dermoid cysts, 310, 321, 324, 328
 Dermoprotease, 704, 705
 Desmoplasia, 590
 Desoxycorticosterone, 41, 103, 213, 265, 442, 444, 445, 446, 546, 547, 572
 Desoxyribose, 374
 Desoxyribosenuclease, 433
 Desquamation (incl. scaling), 186, 203, 216, 247, 377-80, 541, 593, 669, 670, 671, 673, 674, 685, 688; postinflammatory, 234, 377, 489
 Deuterium oxide, 35
 Developmental folds, 3
 Diabetes, 165, 209, 238, 470, 471, 477, 478, 479, 680
 Diascopy, 69
 Diastase, 467
 Diathermy, 264
 Dibenamine, 101, 166, 167, 168
 Dicumarol, 684
 Diethylene glycol, 40, 49
 Diffusion potentials, 9, 10, 13
 Dihydroxyphenolase, 529
 2,3-Dihydroxyphenylalanine, 527
l-3, 4-Dihydroxyphenylalanine; *see* Dopa
d-3, 4-Dihydroxyphenylalanine, 521
dl-3, 4-Dihydroxyphenylalanine, 528, 530
 3,4-Dihydroxyphenylpyruvic acid, 521
 1,3 Diphospho-D-glycerate, 567, 570
 Discrimination: spatial, 121, 125, 126, 136; temporal, 121, 136
 Dismutation, 472, 473
 Disulfide cross-linkages, 341-42, 355, 356-57, 358, 359, 368, 369, 371, 404; closure of, 370, 372
 Diurnal variations: in body temperature, 245; in mitoses, 373, 597; in rate of hair growth, 617-18; in skin surface temperature, 245; in sweat secretion, 238
 Djenkolic acid, 356
 Docosyl alcohol, 321
 Donnan equilibrium, 405, 500, 501
 Dopa, 520, 521, 523, 524, 525, 530, 531, 537, 573
d-Dopa, 521
dl-Dopa, 528, 530
 Dopachrome, 524, 526, 527, 528; reduced, 523, 525
 Dopa-oxidase, 521, 522, 540

- Dopaquinone, 523, 524, 525, 528, 534
- Double pain sensation, 127, 130-31
- Dry skin; *see* Asteatosis
- Dunsthülle, 247
- Dysidrosis, 226
- Dysplasia, multiple ectodermal, 18, 156, 184, 186, 203, 208, 213, 235-37
- Dystrophy of infants, 4
- Eccrine glands: conditioning of, 162, 164, 165, 177, 214; distribution of, 158-61; histochemistry of, 190-92; innervation of, 162-65; number of, 158-61; racial differences in, 158, 160, 161; structure of, 154-55; *see also* Sweat glands; Sweat secretion
- Eccrine sweat, 181, 182, 184, 186, 318; composition of, 201-20; osmotic pressure of, 201, 202; specific gravity of, 201; *see also* Sweat
- "Eczema," 14, 16, 17, 140, 226, 229, 239, 477, 507, 508, 582, 664; *see also* Dermatitis; Atopic dermatitis
- Edema, in protein deficiency, 542, 663, 686
- Egg-white injury, 673
- Ehlers-Danlos syndrome, 6, 450
- Eicosyl alcohol, 321
- Elastase, 411, 422, 438, 448
- Elasticity, 3, 449, 450; of elastin, 412; modulus of, 5, 7
- Elastin (incl. elastic fibers, fibrils, etc.), 341, 391, 393, 395, 410-12, 448, 504, 517, 572, 597, 598, 701; amino acid content of, 411-12; elasticity of, 412; electron microscopy of, 412; swelling of, 412; X-ray diffraction of, 412
- Elastometer, 3, 4
- Elastomucin, 438, 448
- Electrical behavior, 9-25
- Electrical charge, 10, 11, 14
- Electrical double layer, 12, 27, 28
- Electrical pseudo-resistance, 9, 15, 19, 21, 22, 49
- Electrical resistance, 12, 14, 18, 49, 240
- Electrodes, nonpolarizing, 9, 10, 13
- Electrolytes: absorption of, 36-37; in diseased skin, 508-10; and itching, 143; in normal skin, 496-508; *see also* under single ions
- Electro-osmosis, 11, 50, 51
- Electrophoresis, 50-51, 101, 159, 161, 233, 582
- Eleidin, 32, 240, 375, 590, 591
- Elephantiasis, 449
- Emotional sweating; *see* Hyperidrosis, emotional; Sweat secretion, emotional
- Emotional vasomotor reactions: blushing, 75, 91, 189; paling, 85
- Emulsions, 48
- Endothelial cells, capillary, 30, 64, 76, 501, 575
- Enolase, 566, 567, 686
- Enzymes, 564-79; *see also* under single enzymes and groups of enzymes
- Epidermin, 346, 367
- Epidermization, 382
- Epidermolysis bullosa, 699
- Epidermo-protease, 704, 705
- Epinephrine, 47, 49, 77, 80, 81, 87, 88, 101, 102, 103, 105, 140, 161, 166, 167, 168, 172, 183, 190, 261, 467, 521, 526, 546, 547, 582
- Epirenin, 521
- Equivalence of basal cells and prickle cells, 591-93
- Ergosterol, 329, 681
- Ergot alkaloids, 101, 104, 105; dihydro derivative of, 105; ergotamine, 105, 167, 170
- Eriodictyol, 678, 679
- Erysipelas, 508, 639
- Erythematous-urticarial toxic eruptions, 82
- Erythrose peribuccale pigmentaire, 550
- Eserine; *see* Physostigmine
- Essential oils, 49
- Esterases, 575-76, 680
- Estrogens, 41, 46, 102, 103, 177, 302, 373, 375, 380, 382, 398, 441, 453, 547, 548, 549, 626-27, 628, 629, 666
- Ethanol amine, 488
- Ether, 280
- Ethylcarbamate, 632
- Eucalyptol, 49
- Extensibility, 4, 5
- Farmer's skin, 247
- Fats, 43-44, 483-84, 493-94, 565-67, 571, 576, 672; in hair, 313, 317, 328; *see also* Lipids; single compounds
- Fatty acids, 188, 191, 222, 224, 318, 322, 331, 476, 488, 576, 623, 680; antibacterial action of, 318; essential, 318, 484, 670, 681, 684-85; esterified, 312, 314; free, 312, 314, 489; fungistatic action of, 314, 318, 319-20; with odd-numbered carbon chains, 316, 317, 322; unsaturated, 317
- Feathers, 359
- Female sex hormones; *see* Estrogens
- Fibrinogen, 346, 391
- Fibrinoid degeneration, 450-51
- Fibroma and fibrosarcoma, 409
- Filtrate factor, 543, 546
- Flavoprotein yellow enzymes, 665, 668
- Fluorine, 645, 685
- Folic acid, 211, 212, 543, 665, 676, 677; antagonists, 633, 634, 677
- Fordyce disease, 288
- Formalin, 233, 234
- Freckles, 522, 550
- Frostbite, 262
- Fructose 1,6-diphosphate, 567
- Fructose-6-phosphate, 567
- Fumaric acid, 474, 475, 567, 569
- Fungistatic action, 314, 318, 319-20, 645
- Fungus infections, 177, 221, 226, 318, 477-79
- Furo-coumarin, 552
- Galactosamine, acetyl, 422
- Galactose, 44, 396, 421
- Galvanic skin reaction, local, 17, 18, 21-22
- Gases, absorption of, 27, 44-45
- Gaucher's disease, 550
- Gelatin, 393, 394, 404-6, 701
- Glomus bodies, 145
- Glucophosphorylase, 686
- Glucosamine, 422, 424, 428, 440, 448, 471; acetyl, 422; N-acetyl, 422, 423, 431, 572
- Glucosaminidic bonds, 432
- Glucose, 44, 209-10, 396, 421, 422, 427, 428, 436, 466, 467, 566, 570, 704; in skin, 469-79; tolerance test of skin, 470, 477, 478
- Glucose-1-phosphate, 566, 567
- Glucose-6-phosphate, 567, 570, 704
- Glucosidic linkages, 423
- Glucothionic acid, 423
- Glucuronic acid, 422, 423, 424, 427, 428, 431, 572
- β -Glucuronidase, 431, 432
- Glutamic acid, 207, 222, 339, 340, 344, 347, 348, 372, 396, 411, 412
- Glutamine, 207, 347
- Glutaric acid, 316
- Glutathione, 45, 341, 347, 522, 527, 544, 548, 677; oxidized, 347
- Glyceraldehyde-3-phosphate, 567, 570
- Glycerides, 331
- Glycerine, 312, 313, 322, 325-26, 434, 576
- Glycine, 207, 338, 347, 352, 394, 395, 396, 400, 403, 412, 677
- Glycogen, 32, 186, 190, 191, 209, 210, 266, 331, 373, 374, 427, 434, 435, 447, 452, 465-68, 477, 484, 566, 567, 613
- Glycogenolysis, 467
- Glycohistechia, 477
- Glycolysis, 210, 472, 540, 566, 567, 571

- Glycoproteins, 339, 340, 422, 443, 673
 Gold, 531, 645
 Golgi bodies, 330
 Golgi-Mazzoni endings, 123, 147
 Gonadotropins, 441; chorionic, 547
 Granoplasma, 344
 Granuloma: inguinale, 66; pyogenic, 575
 Ground potentials, 10, 15
 Ground substance of connective tissue, 27, 341, 398, 409, 418-64, 471, 701; development of, 446-49; electron microscopy of, 427; functions of, 453; and hormones, 438-42; isoelectric point of, 420; pathologic states of, 449-53
 Growth hormone, 398, 445, 626
 Guanosine, 374
 Gustatory sweating, 178-80
 Gynecomastia, 183, 623
 Hair, 203, 348, 349, 350, 351, 352, 356, 359, 368, 372, 373, 374, 377, 382, 398, 435, 465, 503, 575, 586, 591, 663; ambosexual, 625; axillary, 298, 628-29; beard, 629-30; index, 621; intermediate, 621; medullation of, 621; primary, 619; pubic, 298, 627-28; secondary, 619; sexual, 626; terminal, 625
 Hair cycle, 288, 381, 605-16, 624, 634-36, 639, 674; anagen stage of, 606, 613, 614, 638; artificial induction of, 611-12; catagen stage of, 606, 614, 635; hormonal influences on, 614-15; mosaic pattern of, 606, 612, 614; physiological control of, 614-15; telogen stage of, 606, 613, 614, 635
 Hair disks, 147
 Hair fats, 313, 317, 328
 Hair functions: excretory, 641-45; protective, 584, 602-3; sexual, 603; tactile, 125, 127, 147, 603-4
 Hair growth, 601-61; hormonal influences on, 624-30; in vitro, 622; life-span of, 615-16; nervous influences on, 621-22; nutritional influences on, 622-24; rate of, 616-19
 Hairless mutants, 604
 Hallachrome, 526
 β -Haloalkylamines, 101
 Harderian glands, 673
 Heat conductance, 251-53, 257
 Heat exhaustion, 214-15; syndrome, 270, 282
 Heat loss: by conduction, 247-48; by convection, 247; evaporative, 235-37, 248, 249; by radiation, 245-57
 Heavy metals (and salts), 33, 39, 42-43, 632
 Heliotherapy, 107
 Helium, 32
 Hematidrosis, 187
 Hemochromatosis, 543, 550
 Hemodynamics, 72-79
 Hemoglobin, 516; oxy-, 516, 676; reduced, 516
 Hemoprotein, 340
 Heparin, 432, 435, 448, 449, 451, 630, 632, 633, 634
 Heparinoids, 633, 634, 638
 Hepene, 328
 Herpes: simplex, 99; zoster, 99, 141
 Herter's test, 535
 Hertoghe sign, 627
 Hesperidin, 678, 679
 Hexachlorocyclohexane, 46, 676
 Hexadienol, 168
 Hexanoic acid, 222
 Hexokinase, 466, 566, 567, 570, 571, 704
 Hexosamine, 396, 422, 424, 447, 451; acetyl, 451
 Hexuronic acid, 422, 423
 Hirsutism, 626, 630; *see also* Hypertrichosis
 Histaminase, 96, 574
 Histamine, 42, 47, 48, 50, 52, 53, 83, 84, 90, 91, 94, 101, 108, 138, 142, 143, 172, 180, 234, 445, 449, 453, 467, 550, 582; in antigen-antibody reactions, 97, 98; content of, in skin, 96; desensitization to, 98; destruction of, in skin, 96; liberators, 96; test, 173; theory of triple response, 94-100; in thermal burns, 99
 Histaminemia, 96, 100, 108
 Histidine, 96, 207, 339, 340, 343, 344, 347, 348, 352, 353, 396, 412
 Hofmeister series, 405, 701
 Holocrine glands, 181, 185, 284
 Homogentisic acid, 530, 664
 Homologous series: of acids, 314, 315, 316; of alcohols, 321
 Homoprotocatechuic acid, 521
 Hoofs, 359, 666
 Hormonal influences: on apocrine secretion, 182-83; on collagen, 398-99; on elasticity, 4; on ground substance, 438-42; on hair, 624-30; on keratinization, 380-82; on melanin formation, 542-48; on sebaceous excretion, 295-97; *see also* Sex differences
 Hormones: absorption of, 41; controlling enzymes, 565; local action of, 100
 Hyaluronic acid, 396, 421, 423-25, 429-31, 433-34, 437, 439, 441-42, 444, 448-49, 451-53; protein complex, 427
 Hyaluronidase, 427, 428-33, 435-36, 439-42, 444-45, 448-59, 451-53; assay of, 430-31; inhibitors of, 432
 Hyaluronosulfate, 432
 Hydrocarbons, 322, 328-29
 Hydrocortisone (incl. compound F), 41, 398, 442, 444, 446
 Hydrogen bonds, 357, 358, 372, 380, 392, 400, 404
 Hydrogen sulfide, absorption of, 39, 45
 Hydroquinone, 41, 521, 530, 534, 535; monobenzylether of (also *p*-benzyl-), 530, 534, 535, 551
 Hydroxy acids, 188, 315
 Hydroxyglutamic acid, 339, 354
 Hydroxylysine, 207, 396, 412
p-Hydroxyphenylpyruvic acid, 521
 Hydroxyproline, 207, 338, 347, 349, 351, 395, 396, 400, 403, 409, 411, 412
p-Hydroxypropiofenone, 534, 535
 Hyoscine, 46
 Hyperalgesic states, 131-37
 Hyperemia, reactive, 105-6, 264
 Hyperglycemia, 470
 Hyperidrosis, 165, 178; gustatory, 178-80; localized, 172; perilesionary, 178; *see also* Sweat secretion, emotional
 Hyperkeratinization; *see* Superkeratinization
 Hyperkeratosis, 377, 543, 667, 670, 671, 672, 675, 680, 681, 688
 Hyperpathia, 133
 Hyperpigmentation, 46, 372, 381, 547; postinflammatory, 372, 543; *see also* Pigmentation
 Hyperpituitarism (incl. acromegaly), 165, 625, 626
 Hypersensitivity reactions, 98, 99; tuberculin type, 99, 441
 Hyperthecosis, 627
 Hyperthermia, 236, 252, 257
 Horn, 359, 377, 666
 H-substance, 90, 95, 100, 142
 Hyalinosis, 451
 Hypothyroidism (incl. thyrotoxicosis), 16, 165, 238, 250, 499, 532, 546, 550, 627, 629
 Hypertrichosis, 618, 625, 626, 628, 629, 632; essential, 640-41; lanuginosa, 621; primary, 619-20; *see also* Hirsutism
 Hyperventilation, 75
 Hypoidrosis, 165
 Hypopituitarism, 544, 625, 628, 629
 Hypothyroidism, 16, 238, 250, 628, 629, 680
 Hypotrichosis, 620
 Ichthyosis, 239, 374, 377, 385, 496
 -Id reactions, 226
 Imidazolines, 101
 Immersion foot, 262, 264
 Immunity mechanisms, 452

- Impedance, 15-17
 Incontinentia pigmenti, 549, 551
 Indican, 535
 Indigo, 187
 Indole, 520, 523, 535; 5,6-dihydroxy-dihydroindole-2-carboxylic acid, 523; 5,6-dihydroxyindole, 523, 525, 528, 535; 5,6-dihydroxyindole-2-carboxylic acid, 523, 526; 5,6-dihydroxy-2-methylindole, 526; 5,6-dihydroxy-3-methylindole, 527; 6-hydroxy-5-methyl-3-phenylindole-2-carboxylic acid, 527; -5,6-quinone, 526, 528
 Indoxyl, 187
 Inductive reactance, 14
 Influenza, 639
 Infrabasal horny layer, 29, 32, 373, 375
 Inositol, 211, 212, 543, 622, 623, 675-76
 Insulin, 41, 349, 369, 471
 Intermitotics, 589, 594; time, 595, 596
 Intermedin, 544, 545
 Internuncial neurons, 132, 137, 139
 Intrasympathetic reflexes, 168, 173, 176
 Iodides, 36, 37, 38, 47, 51, 623, 685, 701, 702
 Iodogorgoic acid, 338
 Iodothyroglobulin, 41
 Ion transfer, 50; *see also* Electrophoresis
 Ionic sieves, 10, 28
 Ionizing radiation (incl. X-rays), 145, 477, 532, 535, 550, 613, 624, 630; alopecia from, 634-39; antikeratinizing action of, 638; as cause of pigmentation, 550, 551
 Iontophoresis, 50; *see also* Electrophoresis
 Iron, 191, 192, 216, 530, 544, 565, 568, 575, 623, 642, 643, 685, 686
 Isoacids, 315, 316, 321, 322
 Isoalcohols, 321
 Isocholesterol, 326, 327, 329
 Isocitric acid, 474, 475
 Isocitric dehydrogenase, 475, 568
 Isoelectric point: of amino acids, 340; of collagen, 395; of ground substance, 420; of keratin, 11, 230; of proteins, 29
 Isoleucine, 144, 207, 317, 338, 396, 412, 664
 Isoprel, 167, 530
 Isoprene, 315, 326
 Isoprenoids, 326
 Isovaleric acid, 316
 Itch spots, 123
 Itch stimuli, experimental, 138
 Itching, 121, 129, 130-45, 146, 211, 225; central, 143-44; diurnal variations of, 136, 245; paroxysm of, 140; regional differences in, 137; symptomatic relief of, 144-45
 Itchy skin, 132, 133, 134, 136, 137, 140, 141, 142
 Kallicrein, 434
 Keloid, 409
 α -Keratin, 344, 346, 347, 355, 358, 367, 372
 β -Keratin, 345, 346, 355, 374; cross- β -form, 345
 Keratinization (incl. cornification), 202, 208, 234, 235, 238, 261, 279, 285, 310, 325, 331, 348, 349, 360, 366-90, 475, 486, 487, 488, 489, 543, 586, 590, 613, 632, 633, 634, 637, 638, 664
 Keratins (incl. horny layer, horny material, horny structures, keratin fibers, fibrils, keratin layer), 11-13, 16, 21-22, 29, 31-33, 135, 203, 228-30, 234-35, 239-41, 312, 340-41, 344-47, 349-90, 392, 393, 397, 404, 517, 566, 571, 573, 577, 591, 595, 597, 641, 674; acidity of, 224; amino acid composition of, 349-54; buffering capacity of, 228; classification of, 360; elasticity of, 355, 358; enzymatic digestion of, 359, 573; molecular structure of, 354; *see also* Hair; Nails
 Keratolysis, by lithium bromide, 359, 373
 Keratomalacia, 680, 681
 Keratosis, 378; seborrheic, 574
 Kerosis of Darier, 224
 α -Ketoglutaric acid, 474, 475, 567, 568, 665
 Ketones, 322
 Kitols, 680
 Krause's end-bulb, 122-25, 145, 248
 Kryptosterol, 326
 Kwashiorkor, 542
 Lactic acid (incl. lactate), 101, 210-11, 222, 224, 228, 266, 316, 373, 468, 470, 472, 477, 478, 479, 566, 567, 665
 Lactic dehydrogenase, 567
 Lactogenic hormone, 41
 Langerhans cells, 586
 Lanoceric acid, 315
 Lanolin, 279, 280
 Lanopalmitic acid, 315
 Lanosterol, 326, 327
 Lanthionine, 356
 Lanyl alcohol, 321
 Lathosterol, 323; *see also* Δ^7 -Cholestenol
 Lauric acid, 315
 Lead, 43, 642, 644, 685, 686; tetraethyl, 43, 632
 Lecithin, 434, 623, 676
 Lecithinases, 433
 Leiner's disease, 675
 Lentigo, 541, 550
 Leprosy, 173
 Leschke's syndrome, 550
 Leucenol, 634
 Leucine, 207, 338, 344, 347, 348, 372, 396, 412, 621, 664
d,l-Leucyl-glycyl-glycine, 573
 Leukoderma, 531, 535, 551
 Leukokeratin, 540
 Leukomelanoderma, 545
 Leukoplakia, 378, 688
 Leukotaxine, 100, 103, 442, 573, 704
 Lewisite, 30, 31, 38, 52, 570, 703
 Lichen pilaris, 69, 382
 Lichen planus, 106, 508, 551
 Lichenification, 142, 247
 Light reflection, 245-46
 Linoleic acid, 318, 684, 685
 Linolenic acid, 318, 684, 685
 Lipases, 313, 331, 576
 Lipid cell tumors, 301
 Lipid-soluble substances, 632; absorption of, 27, 37-39, 40, 44; unsaturated, 633-34
 Lipid surface film, 31, 229, 238, 280, 357, 681; composition of, 309-36
 Lipids, 186, 241, 309-36, 483-92, 494; *see also* Fats; single compounds
 Lipofuscin, 191
 α -Lipoic acid, 665
 Lipolysis, 312
 Lipoproteins, 241, 340, 374, 449, 494
 Lithium, 36, 701; keratolytic effect of, 359, 373
 Livedo racemosa, 71
 Livedo reticularis, 71
 α -Lobeline, 169, 173
 Lupus erythematoses, 14, 68, 69, 394, 409, 432, 450, 452, 551, 573, 575, 683
 Lupus vulgaris, 107, 681, 682; *see also* Tuberculosis of skin
 Luteoma, 301, 627
 Lymphomas, 550
 Lyotropic series, 405, 701
 Lysine, 207, 339, 343, 344, 347, 352, 363, 396, 411, 412, 664
 Lysozyme, 673
 Magnesium, 143, 172, 216, 502-3, 504, 505, 506, 510, 530, 541, 641, 642, 685, 686, 688
 Male sex hormones; *see* Testicular hormones
 Malic acid, 474, 567
 Malignant growth, 16, 475, 488, 550, 574, 575, 591, 613; *see also* Melanoma, malignant
 Malignant hypertension, 66
 Mammary glands, 183, 298, 488
 Manganese, 530, 574, 576, 642, 685, 686
 Mast-cell tumors, 449

- Mast cells, 438-39, 448-49; histamine in, 95, 449
 Mecholyl, 49, 50, 101, 159, 169
 Meibomian glands, 288
 Meiwosky phenomenon, 71, 521, 552
 Meissner's corpuscles, 123-25, 147
 Melanin, 517, 518, 519-36, 537, 542, 543, 546, 568, 573, 577, 598, 664, 688; granules, 538-40, 544-45, 551; hormonal factors in formation of, 542-48; N-methyl, 526; neural factors in formation of, 549-50; nutritional factors in formation of, 541-43; pseudo-globulin of, 539; tyrosinase concept of formation of, 523-31
 Melanoblasts, 536, 538
 Melanocyte-stimulating hormone (MSH), 544-47, 549
 Melanocytes, 522, 532, 536-41, 544, 547, 549, 550, 551, 552, 573, 586
 Melanogen, 535, 536
 Melanogenesis, 524, 550; *see also* Melanin
 Melanoid, 516, 518, 519
 Melanoma, malignant, 523, 529, 530, 532, 535, 536, 540, 550
 Melanophores, 545, 546; white, 540
 Melanoprotein, 540
 Melanosis, 535, 542, 543, 544, 546, 547, 551; Riehl's, 545, 550
 Melanuria, 520, 532, 535-36
 Membrane theory of epidermis, 10, 11
 Menopause, 102, 103
 Meralgia paresthetica, 133, 134
 Mercury, 29, 31, 34, 43, 48, 531, 645; ammoniated, 29, 43, 531; bichloride of, 42, 43; calomel, 48; oxide, yellow, 42
 Merkel's disks, 123, 124, 147
 Merocrine glands, 154, 181
 Met, 260
 Metachromasia, 434-41, 443, 446, 449, 451
 Metaplasia, 381, 382
 Metarterioles, 61, 63, 70, 75, 77, 79, 80, 94
 Methionine, 338, 341, 342, 343, 344, 352, 357, 368, 369, 372, 395, 412, 623, 633, 664; sulfone, 347
 2-Methyl-2,4 pentanediol, 40
 Micelle theory, 355
 Microincineration, 375, 376, 502, 506-8
Microsporon audouini, 318-21
Microsporon gypseum, 359
 Miliaria: crystallina, 277; experimental, 277-81; profunda, 272, 276, 277; pustular, 279; rubra, 190, 277
 Mitochondria, 330, 488, 540
 Mitosis, 587-89, 591, 593-95, 597, 612, 615, 633
 Mitotic index, 586-87, 589, 593-94
 Mitotic time, 594
 Mnemodermia, 141
 Modalities, sensory, 121, 125
 Molybdenum, 534, 542
 Mongolian spots, 518, 549, 550
 Monophenolase, 528, 529
 Morphine, 95
 Mottling, 70-72
 Mucins, 284, 422, 426, 429, 431, 436, 438, 441, 448, 450
 Mucoid, 422
 Mucoid degeneration, 450-51
 Mucoitin sulfuric acids, 341
 Muco-oligosaccharidase, 431
 Mucopolysaccharides, 190, 341, 397, 421, 422-28, 431, 436, 437, 443, 447-49, 451, 699, 701
 Mucoproteins, 394, 397, 409, 422, 438, 451, 469
 Mustard gas, 30, 31, 52, 570, 572
 Myelomatosis, 451
 Myoepithelium, 154, 167, 168, 184, 187, 189
 Myosin, 340, 345, 367
 Myristic acid, 314
 Myristic alcohol, 321
 Myxedema, 438, 439, 448, 545, 582, 627, 629; localized, 397, 439, 440
 Nails, 203, 240, 349, 352, 374, 377, 573, 640, 663; brittleness of, 356
 Naphthalenes, chlorinated, 534, 680
 Naphthoquinone, 683-84
 Necrobiosis lipoidica diabetorum, 683
 Nerve endings: encapsulated, 121, 123-25; free, 123-25, 136, 145
 Neurofibroma, 409, 550
 Neurotic excoriations, 139
 Neutralization capacity, 227-30
 Nevi: blue, 518, 550; ophthalmomaxillaris Ota, 549; pigmented, 550; systematized, 549
 Nickel, 530, 642, 685
 Nicotinamide, 104, 566, 568, 668; methyl-, 211, 669
 Nicotine, 42, 169, 171
 Nicotine test, 172, 173
 Nicotinic acid (incl. niacin), 81, 104, 135, 211, 543, 565, 665
 Nicotinic action, 91, 168-73
 Nicotinuric acid, 668
 Niemann-Pick's disease, 550
 Nipple test, 547
 Nitrites, 104
 Nitrogen: ammonia-N, 348; in epidermis, 341; in ground substance, 424; nonprotein, 202, 347-49; total dermal loss of, 202-3, 215; total in skin, 341, 347, 685; urea-N, 348
 Nitrogen gas, 32
 Nitrogen mustards, 52, 535, 570, 576, 633
 Nitrogenous constituents, 337-42
 Nocifensor system, 89, 131
 Nonelectrolytes, 499
 Nonkeratins, 372
 Nor-epinephrine, 77, 85, 86, 88, 101, 103, 162, 163, 167, 445, 547
 Nor-leucine, 338
 Nucleic acids, 540
 Nucleoproteins, 340, 374
 Nucleosidases, 668
 Nucleosides, 374
 Nutritional influences: on fat content, 483-84; on glucose content, 470, 478; on glycogen content, 466; on hair growth, 622-24; on melanin formation, 541-43; on physiology of skin, 601-61; on sebaceous-gland excretion, 302-3
 Nyctalopia, 680
 Obesity, 499
 Occupational dermatoses, 227
 Ochronosis, 550, 664
 Octadecyl alcohol, 321
 Octyl alcohol, 49
 Ointments, 33, 37, 47, 48, 49, 52, 238
 Oleic acid, 318, 483
 Opium, 42
 Ornithine, 204, 207, 574
 Orthoaminobenzoic acid, 534
 Orthobenzoquinone, 524
 Orthodihydroxyphenolase, 530
 Orthophenyldiamine, 534
 Orthoquinones, 523, 528, 530, 533, 534, 668
 Ovomucoids, 422
 Oxaloacetic acid, 473, 474, 475, 566, 567
 Oxalosuccinic acid, 474, 475
 Oxidation-reduction potential; *see* Redox potential
 Oxygen uptake; *see* Respiration
 Pacinian corpuscles, 123, 124, 147
 Pain, 89, 121, 123, 124, 127-29, 130-45, 146; chemical mediation of, 142-45; quantitation of, 134-36
 Palmitic acid, 314, 315, 321, 483
 Pantothenic acid, 41, 211, 212, 371, 541, 542, 565, 566, 568, 623, 624, 672-73, 688
 Pantothenol (incl. panthenol), 41, 624
 Pantoyltaurine, 673
 Papaine, 573
 Papilloma, 575, 613
 Para-aminobenzoic acid, 211, 534, 542, 543, 552, 676

- Para-eleidin, 375
 Parakeratosis, 352, 374, 377, 387, 594, 688
 Paraphenylenediamine, 534
 Parathyroid hormone, 451
 Parathyroid insufficiency, 499
dl-Paredrine, 530
 Passive transfer tests, 97, 98
 Pastes, 52, 239
 Pectinase, 437
 Pellagra, 543, 550, 668, 669, 670; infantile, 542
 Pemphigus, 508, 509, 572, 703, 705
 Penetrasols, 44
 Penetration, 27, 30; of dyes, 11, 12, 31, 33, 51
 Pentanoic acid, 222
 Pentose, 374
 Peptidases, 704; aminoexo-, 573; leucyl-, 686
 Peptide: bond synthesis, 372; linkage, 337
 Peptone, 95, 108, 434
 Periodic acid-Schiff stain, 435-38
 Permeability, 22, 27; capillary, 64, 72, 73, 82, 83, 442; of epidermis to water, 239-41, 261; irreciprocal, 35, 45
 Pernicious anemia, 165
 Perniosis, 67
 Perspiration, insensible; *see* Sweat secretion, insensible; Water loss, insensible
 Phanerogenic zone, 370
 Phase angle, 15, 16, 17
 Phenol oxidases, 672
 Phenols, 39, 42, 210, 277, 374, 530; absorption of, 39-41, 42
 Phenylalanine, 207, 338, 343, 344, 347, 352, 396, 412, 542, 664, 665; 4-methoxy-, 530
 Phenylglucosazon, 471
 Phenylpyruvic acid, 542
 Phenylpyruvic oligophrenia, 542, 664
 Phosphatases, 686, 688; acid, 191, 331; alkaline, 191, 331, 376, 394, 443, 575, 613
 Phosphates, 202, 216, 567, 575, 668; high-energy, 566
 2-Phospho-D-glycerate, 567
 3-Phospho-D-glycerate, 567
 Phosphoenol pyruvate, 567
 Phosphoglucosmutase, 567
 Phosphoglyceromutase, 567
 Phosphohexoisomerase, 567
 Phosphohexokinase, 567
 Phospholipids, 209, 313, 330, 331, 374, 483, 488, 489, 676
 Phosphoproteins, 340
 Phosphorus, 46, 540, 685
 Phosphorylases, 567, 686
 Photosensitization, 543, 550, 669
 Phrynoderma, 680, 681
 Physostigmine, 94, 101, 163, 169
 Pigment spread, 540-41
 Pigmentation, 381, 515-63, 669, 674; postinflammatory, 532, 550
 Pigments, 515-63, 598, 644; blood, 518, 519; iron-containing, 518-19; primary, 516
 Pilifera, 602
 Pilocarpine, 47, 160, 163, 164, 166, 168, 179, 183, 222, 305, 331
 Pilomotion, 82, 88, 174, 189
 Pilomotor: axon reflex, 94, 170, 171, 173; muscles, 161, 167, 189
 Pitressin, 102
 Pituitary, 546, 549, 614; effect of, on hair growth, 625-26, 640; melanocyte-stimulating hormone of, 544-45
 Pituitary-adrenal axis, 211
 Pituitrin, 102
 Pityriasis rosea, 508
 Pityriasis rubra Hebrae, 80
 Pityriasis rubra pilaris, 385
 Plasma-genes, 540
 Pneumonia, 639
 Polarization, 18, 19, 22; currents, 9-13, 19; polarizing membrane of epidermis, 11, 12
 Polyarteritis nodosa, 450
 Polycythemia vera, 68
 Polygalacturonidase, 433
 Polymyxins, 316
 Polyoses, 471
 Polypeptides, 337, 354, 371, 399
 Polysaccharides, 423, 452, 613; *see also under* single compounds and compound groups
 Poral closure, 270-81
 Pore pattern, 33, 34
 Porphyrinuria, 669
 Postmitotics, 589, 594
 Potassium, 11, 13, 14, 37, 47, 143, 172, 202, 212, 214, 215, 234, 501, 502, 504, 505, 508-10, 530, 685, 686, 701
 Prantal, 101, 163
 Precapillary sphincter, 63, 70, 75, 77, 79, 80, 94
 Pre-edema, 499
 Preen gland, 188, 285, 310, 316, 321, 324, 325, 331
 Preferential channel, 61, 63, 70, 74, 253
 Pregnanediol, 641
 Pregnanetrione, 549
 Premotor syndrome, 174
 Pressure, 146, 147
 Prickly heat, 270
 Prisco, 105, 167
 Private climate, 247, 603
 Procaine, 13, 162, 172, 173, 176
 Procollagen, 409, 421, 425, 444, 447, 448, 450
 Proelastin, 448
 Progesterone, 41, 103, 299, 300, 549, 572, 641
 Proline, 207, 338, 340, 347, 348, 395, 396, 400, 403, 409, 412
 Propionic acid, 222
 Proptosis, 440
 Propylene glycol, 33
 Prostigmine, 160
 Protamine, 522
 Proteases, 359, 433, 437, 572-73, 701, 704, 705
 Proteins, 11, 186, 212, 228, 337-40, 370, 392, 485, 499, 565, 566, 571, 572, 573, 590, 623, 624, 670; conjugated, 340-41, 394; deficiency in, 663-65; epidermal, 343-61; fibrous, 340, 344, 354, 499; globular, 340, 344, 347, 368, 369; mucopolysaccharide complex, 325
 Prurigo papules, 139, 140
 Pruritus; *see* Itching
 Pruritus hiemalis, 291, 379
 Pseudohermaphroditism, 629
Pseudomonas tumefaciens, 316
 Pseudo-resistance, galvanic, 9, 15, 19, 21, 22, 49
 Pseudoxanthoma elasticum, 510
 Psoralen, 552
 Psoriasis, 14, 17, 69, 106, 234, 352, 374, 377, 479, 506, 508, 509, 510, 574, 575, 582, 618, 681
 Psychic suggestion and pain, 134
 Psychogalvanic reflex, 13, 16-20, 21, 22
 Psychosomatic excitability, 80
 Psychosomatic influence on inflammatory skin diseases, 91
 Pteroylglutamic acid; *see* Folic acid
 Purines, 374, 577
 Purpura, 518, 679, 684, 686
 Pyridoxal; *see* Pyridoxine
 Pyridoxamine; *see* Pyridoxine
 Pyridoxine, 211, 212, 371, 543, 566, 623, 669-72, 681, 685; 4-pyridoxic acid, 211; 4-desoxy-, 670
 Pyrogallol, 41
 Pyruvic acid (incl. pyruvate), 472, 473, 475, 476, 477, 566, 567, 568, 570, 571, 665, 703
 Pyruvic dehydrogenase, 567, 568, 703
 Quinine, 632
 Quinones, 677
 Radioisotopes, 35, 37, 38, 39, 44, 46, 316, 341, 342, 394, 427, 428, 633
 Radon, 32, 45
 Ranvier nodules, 367
 Red line, 82, 91
 Redox potential, 527-28, 552, 677, 565
 Reflex sweating, 20, 157, 161, 162, 168, 173-77, 183, 248
 Relative humidity of skin, 250
 Relaxin, 437, 440
 Renewal time of epidermis, 594-97
 Resiliency, 3; age differences in, 3

- Resistance: elastic, 4; electrical, 9, 12, 14, 15, 18, 19, 21, 22, 49, 240; nonelastic, 4
- Resorcin, 41
- Respiration, 569-70, 571, 581, 612, 613
- Respiratory quotient, 580
- Reticulin (incl. reticular fibers), 391, 393, 394, 409-10, 421, 437, 438, 446, 447, 701
- Rheumatic fever, 394, 409, 449, 450, 452
- Rheumatic nodules, 408
- Rheumatoid arthritis, 409, 449, 450
- Riboflavin, 212, 543, 565, 566, 568, 577, 623, 665-68; -adenine-dinucleotide, 665, 666; iso-, 666; phenazine analogue, 666; -5-phosphate, 665
- Ribonuclease, 190
- Ribonucleic acid, 190, 375
- Ribose, 374, 668
- Ricketts, 285, 681
- Riehl's melanosis, 545, 550
- Ringworm of the scalp; *see* Tinea capitis
- Rosacea, 256; keratitis, 666, 668
- Rouget cells, 76
- Rubidium, 36, 685
- Ruffini's endings, 123, 124, 145, 248
- Rutin, 678
- Sailor's skin, 247
- Salicylic acid, 40, 41, 47
- Salt; *see* Chlorides; Sodium
- Salt cross-linkages, 355-58, 372, 380, 403-5
- Sarcoidosis, 681
- Scaling; *see* Desquamation
- Scarlet fever, 374
- Scleroderma, 5, 134, 239, 394, 450, 451, 509, 510, 550, 681
- Scleroproteins, 393, 340
- Scopolamine, 46
- Scratch reflex, 139, 140, 604
- Scurvy, 443-44, 451, 543, 679
- Sebaceous cysts, 310, 324
- Sebaceous-gland excretion 284-308, 324, 325, 380; age differences in, 295-307; endocrine influences on, 297-302; mechanism of, 288-94; nutritional influences on, 302-3; regional differences in, 295; sex differences in, 295-307
- Sebaceous glands, 33, 43, 49, 183, 223, 225, 284-308, 382, 484-85, 501, 503, 566, 576, 612, 613, 666, 674, 687, 688; development of, 285-86; free, 288; histochemistry of, 330-31; innervation of, 303-5; structure of, 385-88
- Seborrhea, 290, 291, 300, 630, 631; postencephalitic, 238, 290, 304, 305
- Sebum, 188, 229, 258, 577, 634; casual level of, 289; definition of, 310; emulsification of, 291; excretion rate of, 289, 290, 293, 294; melting point of, 290; replacement sum of, 289; retained level of, 289; saturation level of, 289; specific gravity of, 311; surface tension of, 311-12; viscosity of, 289, 310, 311; *see also* Lipid surface film
- Sekretbechers*, 157
- Selenium, 641, 644
- Self-sterilization, 318
- Semiprivate climate, 247
- Semiquinone, 528
- Senile changes, 450, 496, 509, 510, 628; *see also* Age differences
- Sensations, cutaneous, 120-52; of temperature, 121-24, 127-30, 145-46, 245, 248; of touch and pressure, 121, 123, 124, 128-30, 146-47; *see also* Itching; Pain
- Sensitivity: epicritic, 136; protopathic, 136, 137
- Sensory functions, 120-52, 586
- Sensory impulses: action potentials, 126-28; central pathways of, 128-29; conduction of, 126, 130-31
- Sensory modalities, 121, 124-27
- Sensory receptors, 127, 129, 130, 145, 146, 175, 176, 248, 252
- Sensory spots, 121, 123, 125, 126, 145, 146, 147
- Sensory substance, 94, 143
- Sensory units, 126
- Serine, 207, 208, 338, 347, 369, 395, 396, 403, 412, 677; seryl-glycylglycine, 347
- Serum sickness, 450
- Sex differences: in apocrine glands, 182, 183; in capacitance reactance, 15; in fat content, 483; in lactic acid content of sweat, 211; in pH of sweat, 222-23; in polarization, 12; in rate of hair growth, 617; in sebaceous excretion, 295-97; in sweat secretion, 160, 171, 238; in tensile strength, 7; in water content, 496
- Sex hormones, 284, 298, 445, 597; effect of, on ground substance, 440-41; effect of, on pigmentation, 547-48; *see also* Estrogens; Testicular hormone
- Sexual skin, 382, 440, 441, 447
- Silicons, 641, 642, 685, 686
- Silk protein, 340, 359
- Silver, 531, 642, 685, 686
- Sjögren's syndrome, 681
- Skin cycle, 288, 612
- Skin-nerve units, 172
- Sludges of red cells, 66, 103
- Smallpox, 99
- Smegma, 285, 288, 328
- Sodium, 11, 35-37, 172, 190, 202, 211, 212-15, 265, 266, 280, 498, 500-502, 504-5, 508, 530, 533, 685, 701
- Sodium chloride; *see* Chlorides; Sodium
- Sore magenta tongue, 666
- Spiders, cutaneous arteriolar, 103
- Spines; *see* Bridges, intercellular
- Spodogram, 506
- Spongioplasma, 344
- Spongiosis, 703
- Spreading factor, 429, 432, 433, 434, 441, 442, 445
- Sprue, 550
- Squalene, 312, 327, 328-29
- Stearic acid, 314, 315, 318, 483
- Stein-Leventhal syndrome, 626
- Steroids, 266, 326, 327, 442, 446, 487, 641, 672; *see also under* single compounds
- Sterols, 322-25, 326, 374, 483, 485-88; *see also under* single compounds
- Stilbestrol, 547
- Stomatitis: angular, 674; aphthous, 669
- Stratum: compactum, 32, 377; disjunctum, 31, 377; granulosum, 29, 32, 240, 241, 484, 503, 574, 575, 590, 596, 597; infrabasale, 29, 32, 373, 375; lucidum, 12, 29, 32, 239-41, 488, 507, 590, 597; posteleidinicum, 507; pre-eleidinicum, 507
- Striae distensae, 3, 663
- Stripping of epidermis, 586, 589, 592, 597
- Strontium, 36, 37, 642, 685, 686
- Strychnine, 42, 50
- Succinic acid, 316, 474, 475, 567, 568, 569, 570
- Succinodehydrogenase, 475, 540, 568, 569, 570
- Sucrose, 44
- Sudamina, 277; *see also* Miliaria
- Sulfates, 216, 701
- Sulfhydryl groups (and compounds), 352, 359, 368-72, 381, 385, 387, 531-33, 542-46, 548, 552, 633-34, 638, 641, 644, 703-4
- Sulfocyanates; *see* Thiocyanates
- Sulfonamides, 33, 48, 52
- Sulfur: absorption of, 38, 39; total, in skin, 341-42
- Sunburn reaction, 98, 102, 107, 277, 619; *see also* Dermatitis; Ultraviolet radiation
- Sun-tanning, 272, 534, 552-53; *see also* Hyperpigmentation, postinflammatory
- Superkeratinization (incl. hyperkeratinization), 22, 31, 281, 372, 377, 378, 533
- Sweat, 154-200, 221-23, 235, 236, 294, 502, 577, 602, 603, 663, 688; apocrine, 180-88; axon reflex, 94, 166; cold, 19, 174, 189; composition of, 186-88;

- eccrine, 154-82, 184-86, 188-89; expulsion of, 188-89; pH of, 211, 221-32; reabsorption of, 157, 190, 214
- Sweat chloride/sweating-rate index, 213, 266
- Sweat glands, 154-200, 236, 468, 521, 566, 575, 576, 586, 591, 666; conditioning of, 162, 164-65, 177; fatigue of, 213; histochemistry of, 190-92; structure of, 154-55; *see also* Apocrine glands; Eccrine glands
- Sweat-retention syndrome (incl. closure of sweat-gland pores), 178, 193, 240, 249, 270-83, 378
- Sweat secretion (incl. sweating), 13, 17, 18, 20, 21, 134, 154-200, 248, 582; adrenergic, 166-68; and axon reflex, 94, 166, 168-73, 176; and blood flow, 188-89; and carbon dioxide delivery, 581; in central nervous system diseases, 178; cholinergic, 162-65; cortical center of, 20, 173, 174; diencephalic reflex, 168, 173-77; emotional, 20, 158, 165, 174, 176-77, 183, 212, 236, 251, 305; gustatory, 178-80; insensible, 156-58, 203, 233, 236, 238, 239; intermittent, 157, 190, 203, 205, 208, 209, 214; intrasympathetic reflex, 168, 173, 177; nervous, 168-80; nonnervous, 161-62, 165; periodicity of, 156-58, 189, 238; profuse, 157, 190, 203, 205, 208, 209; racial differences in, 158, 160, 161; reflex, 20, 157, 161, 162, 168, 173-77, 183; sex differences in, 160, 171, 238; spinal reflex, 168
- Sympathin E and I, 90, 167
- Syphilis, 627
- Tattoos, 518
- Taurine, 347
- Telangiectasia, 103, 106; essential, 67
- Temperature of skin surface, 100, 101, 145, 146, 213, 224, 236, 248, 249, 252, 254-60, 290, 377, 552; and alcohol intake, 254-57; and food intake, 245, 254-55
- Tensile strength, 3, 5-7; age differences in, 5; sex differences in, 7
- Tension, 1-3, 4, 5; age differences in, 1
- Terpenes, 49
- Terpenoids, 326
- Testicular hormones (incl. male sex hormones), 103, 298, 299, 300, 302, 547-48, 549, 626, 628, 630, 631
- Testosterone, 41, 103, 298, 299, 300, 301, 382, 398, 441, 442, 453, 627, 628, 629
- Tetrabromotyronine, 634
- Tetracosanoic acid, 314
- Tetraethylammonium, 140, 171, 172, 173
- Tetrasaccharide, 431, 432
- Thalamic syndrome, 133
- Thallium, 542, 613, 624, 627, 630, 632, 633, 638, 645
- Thecoma, 627
- Thermal balance, 106, 244
- Thermal conductance, 251-53, 257
- Thermal contraction and supercontraction, 345, 346, 355
- Thermal gradients, 145, 146, 248, 250, 251-53
- Thermal injury, 252
- Thermal receptors; *see* Sensory receptors
- Thermal stimuli, 177; penetration of, 264; vascular responses to, 100
- Thermoregulation, 174, 180, 244-69; and alcohol intake, 254-57; center of, 244-45; in diseased skin, 261; and food intake, 254-55; role of hair in, 603; by sweating, 248-50; and sweat-retention syndrome, 281-82; by vasomotion, 245, 253-58
- Thiamine, 212, 472, 477, 565, 566, 568, 543, 665; diphospho-, 472, 665; pyri-, 665
- Thiocyanates, 632, 701, 702
- Thioglycerol, 46
- Thioglycolic acid, 46
- Thiouracil, 325
- Thormählen's test, 535, 536
- Threonine, 207, 338, 344, 347, 396, 412, 664
- Thyroid (incl. thyroxine), 37, 302, 533, 545-46, 627, 702
- Thyrotropic hormone, 439, 440
- Tickle, 121, 138, 146
- Tin, 43
- Tinea capitis, 226, 318-21
- Tinea pedis, 318
- Titanium, 642, 685, 686
- Tobacco inhaling, 75, 87, 104
- Tocopherols, 330, 683
- Tonofibrils (incl. epidermal fibrils), 346, 347, 355, 366, 409, 586, 590
- Tonometer, 4
- Tonus potentials, 13-14
- Total base, 504-5
- Touch; *see* Sensations, cutaneous
- Trace metals, 541, 542, 565, 645, 685
- Trench foot, 262, 264
- Tricarboxylic acid cycle of Krebs, 472-75, 566-72, 665
- Trichobezoar, 664
- Trichophyton mentagrophytes*, 318
- Trichophyton purpureum*, 177, 318
- Trichosiderin, 518-19
- Trichostasis lanuginosa, 620
- Trichozoa, 602
- Triglycerides, 44, 312, 325, 326
- Triosephosphate dehydrogenase, 566, 567, 570, 571
- Triple response of Lewis, 94-100, 140
- Triterpenes, 326
- Triterpenoid alcohols, 312, 326-27
- Tritium oxide, 35, 36
- Tropical anidrotic asthenia, 270
- Tryptophane, 207, 338, 343, 344, 349, 352, 353, 372, 395, 396, 412, 425, 453, 518, 542, 664, 669, 670, 677; methyl, 634
- Tubercle bacillus, 316
- Tuberculosis of skin, 498
- Turgor, 4, 441
- Tyndall effect, 517, 519
- Typhoid fever, 639, 640
- Tyramine, 108
- Tyrosinase, 523-31, 540-43, 545-46, 548, 552, 565, 568, 573
- Tyrosine, 207, 338, 340, 343-44, 347-48, 351-53, 372, 395-96, 412, 425, 436, 453, 520, 521, 523-28, 530-31, 533-35, 540, 542, 546, 548, 552, 568, 573, 664, 665, 677; 3-amino-, 530, 533; apo-, 531; dibromo-, 634; di-iodo-, 530; 3-fluoro-, 530, 533; N-acetyl-, 529, 530, 533; N-formyl-, 529, 530, 533; N-methyl-, 526
- Tyrosine antibodies, 531
- Ultraviolet radiation, 22, 97, 107, 108, 131, 247, 277, 477, 520, 524, 528, 532, 534, 547, 550, 552, 582, 603, 611, 668, 681
- "Underglutamic acid," 347
- Unsaponifiable matter, 313, 321, 322
- Uranium, 642
- Urea, 192, 202, 203-5, 207, 347, 348, 374, 574
- Uremia, 165
- Uric acid, 203, 205, 207, 374
- Uricase, 688
- Uronic acid, 422, 451
- Urorosein, 669
- Urtica urens, 95
- Urticaria (incl. urticarial reactions, whealing, wheals), 47, 82-84, 94-100, 142, 703; cholinergic, 97, 576; to cold, 84, 95, 97; factitial, 84, 91, 97; to heat, 84, 97; papular, 97; with pigmentation, 550; pigmentosa, 449, 550; solar, 84, 95, 97, 98
- Vagabonds' disease, 550
- Valine, 207, 316, 338, 344, 347, 348, 372, 396, 411, 412, 664
- Vanadium, 530, 664
- Vascular diseases, peripheral, 102, 105, 250

- Vascular reactions, 79-119; antagonistic, 106, 107; consensual, 106, 107, 108; non-nervous, 79-84
- Vasoconstriction, 18, 104, 174, 189, 582; by central impulses, 85-87; by local reflex, 88; by long reflex, 87, 88; nonnervous, 79; periodical, 67, 75
- Vasodilatation, 104, 134, 189; after alcohol intake, 254-57; antidromic, 89-91; by axon reflex, 89-92, 94, 104, 262; cholinergic, 88, 94, 179; in internal organs in response to cutaneous stimulation, 106-8; after meals, 108, 245, 254-55; nonnervous, 82; rhythmical, 262; by sympathetic fibers, 88-89
- Vellus, 620
- Vernix caseosa, 285
- Vesicants, 571, 576
- Viosterol, 681
- Viscerocutaneous reflex, 256
- Vitamin A, 41, 281, 330, 375, 382, 383, 384, 385, 387, 479, 543, 550, 623, 633, 638, 679-81
- Vitamin B factors, 41, 211, 371, 665-77; *see also under* single factors
- Vitamin B₆; *see* Pyridoxine
- Vitamin B₁₂; *see* Cyanocobalamin
- Vitamin C; *see* Ascorbic acid
- Vitamin D, 41, 107, 285, 310, 329, 330, 681-83
- Vitamin E, 330, 623, 683
- Vitamin H; *see* Biotin
- Vitamin K, 41, 683-84
- Vitamin P, 679
- Vitamins (incl. deficiencies, precursors), 41, 45, 329-30, 541, 543, 565, 566, 571; *see also under* single vitamins
- Vitiligo, 531, 533, 545, 550
- Vogt-Koyanagi syndrome, 531
- Warmth spots, 123, 145, 146
- Warts, 574, 613
- Washable bases, 48, 49
- Water, 493-99, 508-14; absorption of, 35-36; available, 496-97; bound, 499; free, 499; in ground substance, 421; in keratins, 229, 374, 379, 380; in skin, 483, 493-99
- Water loss, insensible, 36, 52, 156, 233-43; total, 236-39; transepidermal, 233
- Wax alcohols, 31, 309, 312, 313
- Waxes, 31
- Weatherbeaten skin, 247
- Wettedness, 250
- Wetting agents, 33, 49
- White line, 79, 80, 82
- Winter itch; *see* Pruritus hiemalis
- Wintergreen oil, 49
- Wool, 349, 351, 354, 357, 359, 371, 372
- Wool fat, 312, 314, 317, 321, 329
- Xanthine oxidase, 577
- X-disease, 680
- Xeroderma pigmentosum, 550
- Xerophthalmia, 680
- Xerosis; *see* Asteatosis
- X-rays; *see* Ionizing radiation
- X-ray diffraction, 344, 391-92, 398, 399-403, 412, 643
- Xylol, 280
- Zinc, 530, 541, 543, 565, 623, 643, 685, 686, 687, 688

Author Index

[Italicized page numbers refer to pages in chapter bibliographies.]

- Aavik, O. R., 163, 184, 185, 186
 Abderhalden, E., 352, 360, 534, 553
 Abelin, I., 484, 485, 490, 499, 510, 645
 Abels, J. C., 676, 688
 Aberg, B., 458
 Abraham, E. E. V., 315, 332
 Abraham, E. P., 669, 692
 Abramson, D. I., 75, 87, 101, 102, 103, 104, 108, 109, 112, 258, 266
 Abramson, H. A., 33, 51, 53, 97, 109
 Abt, A. F., 534, 553
 Ackermann, A., 190, 193
 Adachi, B., 529, 537, 553
 Adams, M. H., 523, 531, 557
 Adams, P. D., 569, 577
 Adams, R., 634, 645
 Adams, W. S., 186, 193, 216, 216
 Adolph, E. F., 175, 213, 216, 265, 266
 Adolph, W. E., 344, 360
 Adrian, E. D., 85, 109, 130, 148
 Ahern, J. J., 64, 109
 Ahlquist, R. P., 86, 102, 109, 119
 Ahmann, C. F., 645
 Ajello, L., 359
 Albanese, A. A., 664, 688
 Albers, H., 668, 690
 Albert, R. E., 157, 238, 241
 Albrieux, A. S., 41, 53, 626, 629, 645
 Albright, F., 625, 626, 628, 629, 640, 645, 650
 Alburn, H. E., 449, 456
 Alcayaga, R., 661, 670, 673, 698
 Aldao, C. N. G., 71, 109
 Alderson, H. E., 478, 480
 Aldrich, C. A., 419, 461
 Aldridge, W. N., 576, 577
 Alex, M., 504, 512
 Alexander, H. L., 577
 Alexander, J., 391, 413, 699, 705
 Alexander, P., 355, 357, 358, 359, 360, 361
 Alexandre, C., 628, 649
 Allen, E., 548, 555
 Allen, S. D., 270, 282
 Allen, W. J., 105, 109
 Alsberg, C. L., 520, 523, 553
 Althausen, T. L., 641, 642, 643, 645
 Altman, A., 542, 553
 Altshuler, C. H., 447, 450, 454
 Alvarez, E., 40, 46, 54
 Alverdes, K., 154, 193
 Amarante, A., 627, 645
 Ambrose, E. J., 401, 413
 Amersbach, J. C., 569, 571, 577
 Ameseder, F., 332
 Amromin, G. D., 444, 463
 Anders, W., 638, 645
 Anderson, D. S., 221, 224, 225, 226, 230, 230
 Anderson, H. K., 93, 115
 Anderson, L. H., 105, 109, 115
 Anderson, R. J., 316, 332
 Andreasen, E., 590, 598
 Andres, A. C., 575, 577
 Andrew, N. V., 590, 598
 Andrew, W., 590, 598
 Andrews, J. C., 664, 677, 688
 Andrus, W. De W., 684, 688
 Angevine, D. M., 447, 450, 454
 Angier, R. B., 676, 689
 Anker, H. S., 487, 490
 Ansbacher, S., 534, 535, 543, 553, 559, 654, 676, 684, 689
 Anslow, W. K., 677, 689
 Anslow, W. P., Jr., 52, 53
 Antinobin, A., 36, 53
 Antopol, W., 645, 670, 689
 Aoki, T., 161, 162, 166, 183, 184, 193
 Apajalahti, L., 627, 645
 Arai, T., 168, 171, 172, 199
 Arey, L. B., 591, 598
 Argyris, T. S., 468, 479
 Arloing, S., 304, 305
 Armin, J., 89, 91
 Armstrong, D., 143
 Arn, K. D., 212, 216
 Arnold, H. L., Jr., 173, 193
 Arnold, L., 228, 230
 Arnow, L. E., 528, 553
 Aron-Brunetiere, R., 300
 Asboe-Hansen, G., 438, 439, 448, 454
 Ashe, W. F., 248, 267, 670, 696
 Asher, L., 107, 109
 Ashford, C. A., 534, 546, 563
 Ashton, N., 445, 454
 Astbury, W. T., 344, 354, 355, 358, 361, 374, 387, 391, 399, 400, 407, 412, 413, 450, 463
 Atchison, G. J., 45, 53
 Atkinson, W. B., 627, 628, 649
 Atwood, E. B., 544, 561
 Au, M. H., 503, 512
 Aubrun, E. A., 622, 645
 Aughey, E., 47, 52, 56
 Austin, W. E., 643, 647
 Avery, O. T., 422, 452, 456, 458
 Axelrod, D. J., 30, 31, 38, 39, 40, 53
 Aykroyd, 381, 387
 Babcock, C., 704, 705, 707
 Baccaredda, A., 645, 645
 Bachman, C., 441, 454
 Badel, M., 96, 99, 112
 Baeder, D. H., 442, 463
 Baehr, G., 450, 458, 459
 Baer, H., 508, 511
 Baer, R. L., 29, 30, 32, 33, 34, 41, 44, 47, 55, 56, 58, 84, 97, 109, 118, 429, 462
 Baumle, W., 217, 227, 228, 230
 Bagchi, K. N., 644, 645, 645
 Bailey, K., 344, 361
 Baisset, E., 470, 479
 Baitsell, G. A., 419, 446, 454
 Baker, B. L., 41, 54, 300, 305, 398, 413, 445, 454, 455, 546, 563, 572, 578, 624, 645, 660
 Baker, L. C., 312, 314, 315, 322, 325, 326, 329, 332
 Baker, R. F., 344, 360
 Balazs, A., 613, 646
 Baldes, F. J., 65, 68, 69, 113
 Baldino, N., 190, 194, 467, 468, 480
 Baldridge, G. D., 446, 454, 584, 599, 699, 706
 Baldwin, E., 337, 342, 361, 473, 475, 479
 Baldwin, I. G., 630, 646
 Bale, W. F., 216, 217, 501, 513
 Balfour, B. M., 443, 461
 Ball, E. G., 528, 553
 Ball, S., 677, 689
 Baló, J., 411, 413, 438, 448, 454
 Banga, I., 411, 413, 438, 448, 454
 Bangham, A. D., 454
 Baptist, M., 625, 651
 Barahal, H. S., 603, 646
 Barail, L. C., 44, 53
 Bárány, R., 143, 148
 Barbaud, J., 643, 651
 Barber, A., 444, 454
 Barber, M., 438, 446, 454
 Barbour, H. G., 193, 498, 512
 Barclay, W. R., 64, 66, 76, 103, 104, 111
 Barcroft, H., 87, 109
 Bardy, H., 92, 109
 Barger, G., 86, 97, 101, 109
 Barker, N. W., 71, 109
 Barksdale, E. E., 84, 109
 Barnett, A., 15, 16, 22
 Barnett, A. J., 166, 167, 193
 Barrett, A. M., 681
 Barritt, J., 352, 361
 Barron, D. H., 89, 109
 Barron, E. S. G., 472, 473, 475, 476, 477, 479, 566, 568, 569,

- 570, 571, 577, 613, 639, 646,
665, 689
- Barscum, G. S., 99, 109
- Bart, A., 228, 230
- Barton, D. S., 103, 109
- Basch, F., 45, 53
- Basler, A., 602, 646
- Battaglini, S., 14, 22
- Baudin, P., 535, 556
- Bauer, H., 434, 454
- Bauer, R. O., 40, 54
- Bauer, W., 425, 426, 429, 431, 462
- Baum, H. M., 542, 560
- Baumann, C. A., 323, 324, 333,
334, 487, 490, 670, 684, 694,
695
- Baumberger, J. P., 699, 701, 705
- Baumgartner, H., 326, 335
- Baxter, H., 398, 413
- Bayless, F., 104, 113
- Bayliss, W. M., 89, 109
- Bazett, H. C., 123, 145, 146, 148,
163, 193, 198, 244, 245, 248,
251, 257, 260, 265, 266, 267,
269, 603, 654
- Beach, E. F., 368, 390
- Beal, P., 84, 89, 95, 97, 98, 109,
117
- Beamer, W. H., 45, 53
- Bean, W. B., 103, 104, 109, 118,
248, 267, 670, 696
- Bear, R. S., 396, 398, 399, 400,
401, 402, 403, 404, 408, 410,
413
- Beaton, L. E., 174, 193
- Beck, W. C., 104, 109
- Becker, J. E., 654
- Becker, S. W., 551, 553, 568, 578,
618, 646
- Becker, S. W., Jr., 520, 523, 532,
536, 538, 550, 551, 553, 555
- Becker, W. H., 245, 267
- Beecher, H., 516, 562
- Beecher, H. K., 76, 109, 120, 135,
148, 150
- Beek, C. H., 625, 628, 629, 630,
646
- Beek, J., Jr., 396, 407, 413
- Beer, R. J. S., 527, 553
- Beeson, B. B., 622, 646
- Begany, A. J., 442, 449, 456, 463
- Begemann, H., 451, 458
- Behnke, A. R., 32, 53
- Behrendt, H., 221, 223, 226, 230
- Behring, H. von, 323, 332, 335,
491
- Behrman, H. T., 624, 646
- Beiler, J. M., 96, 116
- Belding, A. S., 265, 268
- Belinfante, A. J. G., 632, 646
- Bell, R., 531, 553
- Bellamy, L. J., 327, 332
- Beloff, A., 573, 577, 704, 705
- Benditt, E. P., 435, 436, 437, 441,
443, 454, 456, 457, 462
- Benedict, C. G., 238, 241
- Benedict, F. G., 100, 109, 233,
238, 241, 258, 266, 603, 646
- Benend, W., 327, 336
- Bengston, B. N., 640, 646
- Bennett, S., 166, 183, 196
- Bensley, S. H., 419, 435, 443, 454
- Benson, S., 101, 111
- Benyamovich, E. B., 666, 694
- Berenblum, I., 569, 577
- Berenson, G. S., 240, 241
- Bergeim, O., 204, 207, 211, 216,
217, 222, 223, 225, 230, 624,
655, 676, 694
- Bergental, D. M., 547, 553
- Berger, A. R., 245, 267
- Bergman, A., 632, 646
- Bergman, H. C., 640, 650
- Bergmann, M., 337, 340, 342,
361, 395, 413
- Bergstrand, H., 627, 652
- Bergstrom, S., 448, 458
- Bergy, C. A., 342, 356, 361
- Berman, L., 644, 647
- Bernard, C., 373, 387
- Bernhard, O., 107, 109
- Bernheim, F., 531, 553
- Bernheim, M. L. C., 531, 553
- Bernstein, C., Jr., 685, 691
- Bernstein, E. T., 534, 553
- Berry, E. L., 102, 114
- Berthold, 617, 646
- Bertier, L., 632, 646
- Bertrand, G., 523, 553, 554
- Bessey, O. A., 382, 390, 670, 680,
698
- Best, C. H., 86, 189, 193, 264,
266, 574, 577, 663, 689
- Best, W. R., 646
- Bethard, W. F., 216
- Bethe, A., 10, 22
- Beutner, R., 11, 23
- Bevans, M., 445, 455
- Bevelander, G., 613, 652
- Beveridge, J. M. R., 351, 353,
354, 361, 363
- Bibler, W. G., 501, 511
- Bickford, R. G., 132, 133, 137,
146, 148, 168, 172, 193
- Bidwell, E., 397, 413, 433, 454,
573, 575, 577
- Biedermann, W., 284, 305, 584,
598
- Bierman, W., 202, 210, 211, 217,
222, 228, 229, 230, 318, 335
- Biese, J. J., 575, 577
- Biese, M. M., 575, 577
- Bigelow, N., 131, 135, 148
- Bignardi, C., 434, 454
- Billingham, R. E., 536, 540, 553
- Billingsley, P. R., 85, 116
- Binckley, G. W., 669, 692
- Bing, H. I., 122, 123, 142, 148
- Birch, T. W., 670, 685, 689
- Birkeland, J. M., 432, 462
- Bischler, A., 38, 46, 53
- Bischoff, F., 670, 689
- Bishop, G. H., 120, 121, 123, 125,
126, 127, 128, 129, 130, 136,
138, 139, 142, 146, 147, 148
- Bishop, P. M. F., 641, 646
- Bissell, G. W., 628, 629, 640, 645,
646
- Bissonnette, T. H., 381, 387, 611,
614, 646
- Bittinger, I., 684, 694
- Bizzozero, E., 703, 705
- Black, A., 655, 674, 681, 694, 696
- Blackberg, S. N., 535, 553
- Blackstock, V., 668, 697
- Blackwood, B., 516, 562
- Blaisdell, R. K., 262, 266
- Bland, E. F., 78, 113
- Blaney, A. E., 509, 512
- Blank, H., 344, 362
- Blank, I. H., 10, 19, 23, 222, 230,
379, 380, 387, 597, 598, 699,
707
- Blatt, A. H., 337, 342, 362
- Bliss, A. R., 47, 53
- Blix, G., 424, 426, 454
- Blizzard, W. L., 604, 648
- Bloch, B., 295, 299, 305, 520, 523,
531, 537, 546, 553, 554
- Bloch, E. H., 66, 103, 114
- Bloch, K., 327, 328, 333, 336,
487, 490
- Block, M., 264, 266
- Block, R. J., 342, 342, 343, 351,
352, 353, 354, 361
- Block, W. D., 621, 646
- Bloom, F., 449, 454, 461
- Bloom, F. H., 449, 461
- Bloom, W., 446, 460
- Bloor, W. R., 488, 490
- Blum, H. F., 84, 109, 552, 554
- Blumensaat, C., 611, 646
- Blunt, J. W., 444, 446, 454, 462
- Bluntschli, H. J., 105, 110
- Boas, M. A., 673, 689
- Boas, N. F., 440, 441, 447, 454,
455, 459
- Bocher, C. A., 632, 634, 655
- Bock, F., 323, 329, 336
- Bocquillon, P., 632, 646
- Bodo, R. C., 479
- Boehm, F., 535, 554
- Boeke, J., 303, 305
- Boelter, M. D. D., 686, 689
- Bogue, R. H., 404, 410, 413
- Bohnenkamp, H., 246, 266
- Bohnstedt, R. M., 471, 480
- Bohren, B. B., 518
- Bohstedt, G., 648
- Bolduan, O. E., 396, 413
- Bolliger, A., 203, 208, 210, 217,
348, 361, 372, 374, 387, 465,
475, 479
- Bolling, D., 342, 342, 361
- Bolyard, M. N., 328, 336
- Bommer, S., 143, 148
- Boncoddo, N., 489, 491
- Bonner, O., 670, 695
- Bonniger, M., 1, 7
- Bonting, S. J. L., Jr., 341, 342,
342, 343, 361
- Booth, G., 108, 110
- Borasky, R., 407, 416
- Borberg, A., 646
- Borchardt, W., 215, 217
- Bordley, J., 67, 110

- Bordley, J. E., 20, 23, 67, 72, 110, 112
 Borghi, B., 348, 361
 Borgstrom, P., 202, 203, 217
 Boring, E. G., 120, 148
 Bornstein, A., 493, 494, 510
 Boruttau, H., 16, 23
 Bost, R. W., 202, 203, 217
 Bostroem, E., 590, 598
 Bourbon, P., 646
 Bourne, G. H., 455
 Bourquelet, E., 523, 554
 Bowes, J. H., 394, 395, 396, 397, 409, 411, 412, 413
 Bowness, J. M., 541, 554
 Boyland, F., 429, 455
 Boynton, M. W., 46, 54, 381, 387, 547, 548, 554
 Bozer, J. M., 544, 555
 Braccini, C., 448, 455
 Brack, W., 105, 110, 140, 142, 148
 Bradfield, J. R. G., 373, 387
 Bradford, F. H., 138, 139, 144, 148
 Brandman, O., 264, 268
 Braun, H. A., 633, 646
 Bray, B. M., 641, 646
 Brenner, S., 681, 689
 Breslauer, F., 92, 110
 Brewer, D. B., 438, 455
 Brewer, J. J., 103, 110
 Bridges, M. E., 680
 Bridgman, R. M., 572, 578
 Briggs, A. P., 674, 696, 697
 Briggs, G. M., 543, 558
 Bright, E. M., 622, 647
 Brigon, A., 623, 652
 Brink, N. G., 677, 689, 695
 Brinkhous, K. M., 684, 696
 Brisou, J., 397, 413, 573, 577
 Brobeck, J. R., 174, 197
 Brockman, J. A., Jr., 677, 689
 Brodbury, J. G., 302, 308
 Broders, A. C., 587
 Bronk, D. W., 85, 109
 Broquist, H. P., 677, 689
 Broster, L. R., 646
 Brown, A., 675, 689
 Brown, A. E., 358, 362
 Brown, E. V., 40, 53
 Brown, G. B., 516, 561
 Brown, G. E., 95, 114
 Brown, H., 341, 342, 352, 363, 494, 501, 503, 504, 505, 509, 510, 512, 623, 646, 670, 686, 693, 698
 Brown, P. K., 634, 660
 Brown, W. H., 611, 646
 Browne, J. S. L., 99, 117
 Brown-Séquard, 106, 110
 Brožek, J., 4, 7
 Bruce, A. N., 92, 94, 110
 Brunner, M. J., 356, 358, 361, 397, 416, 646
 Brunsting, H. A., 183, 193
 Brunsting, L. A., 71, 116, 516, 518, 554, 562, 681, 689
 Brustier, V., 646
 Bryant, J. E., 202, 215, 216, 220
 Buechler, P. R., 508, 514
 Bülbring, E., 86, 88, 110
 Bürger, M., 485, 490, 495, 510
 Bürgi, E., 27, 34, 36, 38, 39, 40, 42, 53
 Büttner, K., 267
 Buguward, L., 470, 479
 Bujadoux, A., 304, 306
 Bulliard, H., 352, 360, 362, 370, 372, 388, 615, 616, 618, 647
 Bullough, W. S., 290, 302, 306, 373, 387, 595, 597, 598, 599
 Bu'lock, J. D., 526, 554
 Bunak, W. W., 518, 554
 Bunting, H., 186, 189, 190, 191, 192, 193, 435, 438, 441, 447, 455, 456, 464
 Burch, G., 498, 510
 Burch, G. E., 29, 31, 53, 239, 240, 241, 241, 243, 510
 Burckhardt, W., 217, 227, 228, 229, 230, 261, 267, 469, 471, 481
 Burgess, J. F., 683, 689
 Burk, D., 539, 540, 561, 563
 Burlet, E., 41, 53
 Burn, J. H., 80, 86, 88, 110, 549, 554
 Burnet, F. M., 431, 455
 Burns, E. L., 381, 389
 Burr, G. O., 318, 332, 684, 685, 689
 Burr, M. M., 318, 332, 684, 685, 689
 Burrows, H., 373, 382, 387
 Burstein, L. S., 485, 487, 491, 577, 579
 Burtenshaw, J. M. L., 318, 332
 Burton, A. C., 74, 75, 76, 85, 87, 108, 110, 251, 253, 258, 260, 267
 Burton, H., 526, 554
 Buschke, A., 631, 633, 644, 647
 Buschke, W., 183, 193, 302, 306, 664, 666, 688, 698
 Bush, I. E., 624, 647
 Butcher, E. O., 293, 294, 295, 306, 310, 311, 312, 324, 332, 381, 387, 546, 554, 606, 611, 612, 613, 614, 615, 647, 652
 Butler, R. E., 666, 695
 Butt, A. J., 451, 455
 Butt, H. R., 677, 689
 Butterworth, T., 618, 656
 Bychkov, S. M., 425, 455
 Byer, J., 87, 118
 Byrom, F. B., 438, 455
 Bywaters, E. G. L., 442, 449, 455, 456
 Cahill, J. A., 105, 119
 Caldine, D., 647
 Caldwell, A. L., 364
 Calkins, E., 523, 528, 529, 530, 558
 Callaway, J. L., 640, 647
 Calvery, H. O., 27, 32, 34, 35, 38, 40, 41, 43, 44, 50, 54, 633, 646
 Cameron, G., 538, 556
 Camp, E., 318
 Campbell, C. J., 81, 91, 94, 115
 Campbell, J. A., 580, 583
 Cannon, P. R., 664, 689
 Cannon, W. B., 86, 110, 163, 167, 193, 622, 647
 Cantarow, A., 531, 534, 560
 Carey, B. W., Jr., 469, 471, 481
 Carleton, A. A., 587
 Carmichael, E. A., 101, 110, 179, 199
 Carpenter, R., 202, 215, 216, 220
 Carretero, R., 670
 Carrié, A. B. D., 355, 361
 Carrié, C., 210, 213, 217, 313, 324, 332, 478, 479, 490
 Carrier, E. B., 67, 72, 82, 104, 110, 116
 Carruthers, C., 348, 364, 475, 476, 480, 485, 490, 491, 494, 501, 502, 503, 505, 510, 511, 513, 514, 569, 575, 577, 579, 699, 707
 Carter, A. S., 633, 656
 Carter, J., 404, 405, 417
 Cartwright, G. E., 670, 689, 698
 Casazza, R., 645, 647
 Case, R. A. M., 46, 54
 Cason, J. E., 461
 Caspe, I. J., 643, 647
 Cassel, J. M., 395, 397, 413
 Castellino, P. G., 359, 361
 Castle, W. E., 604, 647
 Castor, C. W., 41, 54, 398, 413, 445, 455
 Castorina, G., 638, 659
 Catch, J. R., 316, 332
 Catchpole, H. R., 397, 414, 433, 437, 440, 441, 442, 443, 447, 450, 451, 452, 455, 457, 461, 699, 701, 706
 Cater, D. B., 331
 Caujolle, F., 45, 57
 Cavallero, C., 448, 455
 Cavier, R., 27, 38, 41, 46, 49, 59
 Cawley, E. P., 547, 563
 Ceccaldi, P. F., 37, 38, 58
 Ceccarini, F., 486, 491
 Cedar, E. T., 681, 689
 Cerecedo, L. R., 685, 697
 Cerutti, P., 188, 197, 231, 305, 306, 611, 647
 Cervino, J. M., 545, 560, 627, 655
 Chace, R. R., 632, 656
 Chaffee, E., 423, 424, 425, 429, 460
 Chaikoff, I. L., 485, 487, 491, 577, 579, 688, 694, 696
 Chain, E., 423, 429, 432, 441, 455, 569, 577
 Chalmers, I. M., 160, 164, 165, 166, 167, 168, 193
 Chambers, A. H., 45, 54
 Chambers, G. K., 605, 647

- Chambers, R., 61, 63, 64, 67, 72, 73, 75, 76, 83, 110, 345, 361, 452, 464, 686, 689
 Champetier, G., 344, 362, 367, 387, 388
 Champy, C., 304, 306
 Chance, G. Q., 134, 149
 Chang, H., 633, 647
 Chapman, W. P., 140, 151
 Charles, D. R., 523, 554
 Charpy, M. J., 683, 690
 Chase, H. B., 186, 191, 192, 197, 352, 373, 389, 435, 460, 465, 480, 535, 554, 611, 612, 613, 635, 647, 654
 Chase, W. E., 664, 689
 Chavarria, A. P., 624, 647
 Cheetham, H. D., 669, 694
 Chen, T. T., 528, 553
 Cherbuliez, E., 405, 413
 Chernikov, M. P., 409, 413
 Chieffi, M., 4, 7, 629, 647
 Chiego, B., 356, 362
 Childs, A., 500, 501, 510
 Chittenden, R. H., 668, 690
 Chlopin, N. G., 385, 386, 387, 591, 599
 Christ, J., 621, 647
 Christian, J. E., 35, 56, 289, 308
 Christian, W., 665, 668, 697
 Chrometzka, F., 233, 236, 238, 241
 Chu, F. T., 382, 388, 680, 691
 Clark, D., 131, 148
 Clark, E. L., 64, 66, 67, 76, 78, 110, 419, 455
 Clark, E. R., 64, 66, 67, 76, 77, 78, 110, 111, 419, 455
 Clark, F. H., 604, 648
 Clark, G., 174, 194
 Clark, G. L., 399, 404, 405, 413
 Clark, H. E., 214, 217
 Clark, W. G., 32, 48, 49, 51, 58
 Clarke, G. E., 681, 697
 Claude, A., 429, 433, 455
 Claushen, A., 166, 194
 Clavelin, P., 643, 651
 Clay, R. C., 621, 648
 Clayton, B. E., 444, 455
 Clemo, G. R., 526, 527, 554
 Cline, J. K., 665, 690
 Clisby, K. H., 532, 561
 Cloetens, R., 688, 690
 Clowes, G. H. A., 364
 Cochran, K. W., 576, 578
 Code, C. F., 96, 99, 111
 Cohen, M. H., 362
 Cohen, M. R., 626, 653, 658
 Cohen, T., 101, 111
 Cohn, E. J., 337, 342, 362, 368, 387
 Cole, D. F., 499, 510
 Cole, H. N., 42, 43, 54
 Cole, K. S., 15, 23
 Colle, J., 15, 23
 Collins, H. H., 611, 648
 Collip, S. B., 441, 454
 Comar, C. L., 542, 554
 Combes, F. C., 41, 54
 Comeau, W. J., 97, 113, 576, 578
 Comél, M., 541, 554
 Comstock, C. R., Jr., 32, 56
 Conant, J. B., 337, 342, 362
 Conn, J. W., 190, 194, 213, 214, 217, 265, 266, 267
 Connors, C. A., 666, 690
 Consden, R., 450, 457
 Consolazio, F. C., 213, 214, 218, 265, 268
 Consolazio, W. V., 509, 514
 Conti, O., 545, 560
 Cook, B. B., 543, 559
 Cook, C., 445, 454
 Cook, E. S., 569, 571, 577
 Cook, K., 621, 648
 Cooke, R. E., 215, 218
 Coon, J. M., 80, 88, 90, 94, 111, 117, 161, 163, 164, 167, 168, 169, 170, 171, 172, 173, 176, 189, 194, 198
 Coon, W. W., 444, 463
 Coonin, A., 310, 311, 312, 332
 Coons, A. H., 452, 455
 Cooper, Z. K., 587, 591, 597, 599, 600, 626, 627, 633, 648
 Cooperman, J. M., 674, 690
 Cope, O., 573, 579, 704, 707
 Cordero-Carvajal, E., 542, 560, 624, 647
 Corey, R. B., 400, 401, 416
 Cori, C. F., 467, 479
 Cori, G. T., 467, 479
 Corkill, B., 467, 480
 Cormia, F. E., 138, 140, 142, 144, 145, 148
 Cornbleet, T., 139, 140, 148, 151, 183, 194, 204, 207, 211, 216, 217, 222, 223, 225, 230, 330, 332, 466, 467, 469, 470, 471, 480, 500, 501, 503, 504, 505, 510, 543, 554, 624, 650, 685, 690
 Cosacresco, A., 630, 648
 Costa, E., 572, 578
 Costello, C. J., 490
 Cotter, L., 534, 554
 Cotton, T. F., 92, 111
 Coujard, A., 303, 306
 Coujard-Champy, C., 303, 306
 Coursin, D. B., 670
 Cowdry, E. V., 1, 4, 7, 8, 347, 364, 485, 491, 503, 511, 514, 584, 587, 589, 599, 616, 659, 699, 701, 705
 Cowgill, G. R., 673, 694
 Cowie, D. B., 64, 111
 Craft, W. A., 604, 648
 Craig, W. McK., 71, 109, 253, 266, 269
 Cramer, E., 213, 217
 Creditor, M. C., 445, 455
 Crew, F. A. E., 604, 648
 Crigler, J. F., 641, 660, 661
 Cronin, T. D., 40, 54
 Cronvich, J., 498, 510
 Crowley, N., 432, 455
 Csillag, J., 618, 648
 Cullen, C. H., 134, 149
 Cullumbine, H., 30, 31, 38, 52, 54, 573, 578, 704, 705, 705
 Cunha, T. J., 648
 Cunningham, I. J., 648
 Curtis, A. C., 547, 563, 681, 690
 Curtis, R. G., 327, 332
 Cutner, M., 33, 54
 Cutting, C. C., 622, 652
 Cuyler, W. K., 625, 651
 Czetsch-Lindenwald, H. von, 223, 230
 Dähn, W., 503, 511
 Daft, F. S., 676, 684, 690, 693
 Dale, H. H., 85, 90, 94, 97, 101, 109, 111, 162, 163, 174, 194
 Dallenbach, K. M., 120, 149
 Dalton, A. J., 538, 559
 D'Alton, C. J., 238
 Dam, H., 684, 690
 Damm, G., 648
 Danforth, C. H., 604, 606, 611, 615, 616, 618, 620, 625, 640, 648
 Daniel, D., 323, 332
 Danielli, J. F., 394, 413, 414, 443, 455, 456, 575, 578
 Danielson, I. S., 495, 513
 Dann, F. P., 543, 556
 Dann, W. J., 669, 690
 Danneel, R., 532, 561
 Darling, R. C., 214, 217, 238
 Darmstädter, L., 315, 321, 332
 Darrow, C. W., 18, 19, 20, 23
 Darrow, D. C., 215, 218, 498, 500, 502, 512
 Dastré, 106, 111
 Davenport, H. K., 150
 David, L. T., 604, 611, 612, 648
 Davies, D. F., 214, 217
 Davis, G. K., 541, 542, 554, 562, 672, 696
 Davis, M., 665, 679, 694
 Davis, M. E., 46, 54, 381, 387, 547, 548, 554
 Davison, H. G., 543, 559
 Dawbarn, M., 369, 387
 Dawes, B., 544, 554
 Dawson, C. R., 528, 530, 559, 560
 Dawson, H. L., 614, 648
 Dawson, J. W., 535, 554
 Dawson, M. H., 423, 424, 429, 458, 460
 Day, H. G., 648, 650, 655, 688, 690, 691
 Day, P. L., 676, 690
 Day, R., 245, 266
 Day, T. D., 407, 414, 420, 421, 425, 427, 438, 453, 455, 701, 706
 Debidour-Monrad, H., 618, 648
 De Boer, B., 493, 498, 511
 DeBusk, B. G., 665
 Deckert, W., 642, 643, 657
 De Coulon, A., 523, 554
 De Coursey, E., 537, 558, 639, 653
 Deering, R., 169, 176, 188, 189, 198

- Deichmann, W. B., 40, 54
 Dekanski, J., 83, 96, 100, 111
 De Lalla, V., Jr., 83, 111
 Delaunay, A., 438, 446, 454
 Delker, L. D., 664
 Deme, S., 305, 307, 330, 334
 Dempsey, E. W., 186, 189, 190, 191, 192, 193, 435, 447, 464
 Dempsey, M., 406, 407, 415, 436, 455, 456
 Denkwalter, R. G., 667, 692
 Denss, R., 326, 335
 Denton, C. R., 534, 535, 552, 554
 Depisch, F., 470, 481
 Derby, S., 650, 685, 691
 Derksen, J. C., 344, 354, 362, 367, 388
 Dérobert, L., 641, 642, 643, 648, 651
 De Robertis, E., 410, 412, 414
 De Rose, A. F., 677, 691
 Dervinis, A., 442, 463
 De Sanctis, 316, 332
 Desanti, E., 470, 480
 Deuel, H. J., Jr., 649
 De Vaughn, N. M., 674, 696, 697
 De Wolf, H. F., 42, 43, 54
 Dick, J. C., 1, 2, 4, 7, 410, 414
 Dickens, F., 475, 476, 480
 Dickinson, S., 374, 387
 Diecke, S. H., 532, 554, 606, 614, 648, 649
 Diehl, F., 16, 23
 Dill, D. B., 202, 203, 210, 211, 215, 217, 222, 230, 265, 267, 268, 269
 Dillaha, C. J., 640, 649
 Dillon, J. B., 87, 111
 Dimick, M. K., 543, 555
 Dimter, A., 321, 328, 329, 332
 Dinischiotu, G. T., 630, 648
 Diósy, A., 162, 171, 176, 195
 Di Palma, J. R., 79, 80, 102, 103, 106, 111, 116, 117, 630, 656
 Dixon, A. S. J., 442, 456
 Dixon, H. A., 535, 555
 Dixon, M., 566, 569, 570, 571, 578, 704, 706
 Dobrovolskaia-Zavadskaia, N., 520, 535, 555
 Dochez, A. R., 452, 456
 Dodds, E. C., 438, 456
 Dörfel, J., 498, 501, 503, 504, 505, 509, 511
 Doerks, G., 4, 7
 Dole, V. P., 204, 219
 Donal, J. S., 45, 55
 Donaldson, E. C., 640, 650
 Donegan, J., 444, 462
 Donnet, V., 88, 115
 Dorée, C., 326, 327, 332
 Dorfman, A., 421, 425, 428, 431, 432, 441, 452, 454, 456, 459, 462
 Dougherty, F., 444, 456
 Dougherty, I., 169, 176, 188, 189, 198
 Douglass, M., 630, 649
 Doupe, J., 74, 86, 100, 111, 119, 134, 149, 168, 172, 200, 305, 622, 649
 Dowling, G. B., 683, 690
 Downman, C. B. B., 87, 111
 Draeseke, L., 639, 654
 Draganesco, S., 630, 648
 Dragstedt, C. A., 96, 97, 111
 Draize, J. H., 27, 32, 34, 35, 38, 40, 41, 43, 44, 45, 46, 50, 54, 55, 56, 223, 224, 230
 Drastich, L., 265, 269
 Drennan, J. M., 449, 456
 Drew, A. H., 385, 388
 Dreyfus, G., 628, 649
 Driver, R. L., 49, 56
 Droegemueller, W. H., 150
 Drummond, J. C., 312, 314, 315, 322, 325, 326, 329, 332
 Dry, F. W., 606, 649
 Dry, R. M. L., 143
 Dubiski, J., 363
 Du Bois, E. F., 160, 194, 238, 242, 243, 244, 247, 249, 256, 257, 267
 Du Bois, K. P., 531, 555, 576, 578
 Du Bois, R. O., 382, 390
 Dubos, R., 429, 460
 Du Buy, H., 540, 563
 Duemling, W. W., 44, 49, 54
 Dünner, M., 229, 230, 289, 291, 292, 294, 295, 306, 308
 Duffield, J. A., 526, 554
 Duffy, E., 20, 23
 Duggins, O. H., 621, 649, 659
 Dun, F. T., 130, 149
 Dunihue, F. W., 394, 416
 Duntley, S. Q., 103, 111, 516, 517, 518, 519, 522, 552, 555
 Dupertuis, C. W., 627, 628, 649
 Duran-Reynals, F., 428, 429, 434, 441, 447, 455, 456
 Durham, F. M., 523, 555
 Durward, A., 605, 612, 615, 649
 Du Shane, G. P., 538, 555
 Duspiva, F., 359, 362, 363
 Dutcher, T. F., 519, 555, 643, 649
 Duthie, D. A., 450, 461
 Duthie, E. S., 423, 429, 432, 441, 455
 Duthie, J. J. R., 453, 462
 Du Vigneaud, V., 673, 690
 Duxbury, F. K., 526, 527, 554
 Eakin, R. E., 673, 690
 Earland, C., 355, 360
 Eaton, B. C., 19
 Eaton, M. W., 617, 649
 Eaton, P., 617, 649
 Eaves, C., 426, 427
 Ebbecke, U., 21, 23, 79, 80, 82, 111
 Ebert, M. H., 681, 690
 Ebert, R. H., 64, 66, 76, 103, 109, 111, 117, 445, 456
 Ebling, F. J., 290, 297, 298, 300, 301, 302, 303, 306, 597
 Ebstein, E., 631, 649
 Eckhardt, R. E., 666, 670, 690, 692
 Eckstein, A., 50, 54
 Eckstein, H. C., 303, 306, 324, 325, 332, 344, 352, 353, 362, 344, 486, 490
 Eckstrom, P. E., Jr., 362
 Edelhausen, J. H., 677, 697
 Ederstrom, H. E., 159, 189, 198
 Edsall, J. T., 337, 342, 362, 368, 387
 Edwards, E. A., 103, 111, 516, 517, 518, 519, 522, 552, 555
 Edwards, H. T., 202, 203, 210, 211, 215, 217, 222, 230, 265, 269
 Edwards, L. D., 289, 308
 Edwards, W., 120, 126, 135, 149
 Edwards, W. M., 633, 655
 Eggleston, W. G. E., 644, 649
 Ehrenwald, H., 136, 149
 Ehrhardt, K., 41, 54
 Ehrich, W. E., 449, 450, 451, 452, 456
 Eichelberger, L., 341, 342, 362, 493, 494, 495, 497, 498, 500, 501, 502, 503, 504, 509, 510, 511
 Eichenlaub, F. J., 591, 599
 Eichholz, E., 611, 649
 Eichna, L. W., 72, 112, 245, 248, 265, 267
 Eilers, H., 499, 511
 Eimer, K., 233, 242
 Einbinder, J., 397, 414, 425, 456
 Einhauser, M., 534, 561
 Eisele, C., 341, 342
 Eisele, C. W., 493, 494, 497, 498, 500, 501, 503, 504, 505, 509, 511
 Eisen, A. Z., 352
 Eisenbergh, L., 245, 266
 Eisner, H., 48, 54
 Eldredge, J., 216
 Elftman, H., 627, 628, 649
 Eliot, T. S., 66, 103, 114
 Eller, J. J., 41, 43, 47, 54
 Ellinger, F., 96, 112
 Elliot, A. H., 67, 112
 Elliot, R., 577
 Elliott, A., 401, 413
 Elliott, G. H., 355, 364
 Elliott, J. A., Jr., 37, 54
 Ellis, G. H., 541, 562, 658
 Ellis, L. B., 72, 90, 101, 112, 118
 Ellis, W. J., 372, 388, 643, 649
 Elöd, E., 351, 358, 359, 362
 Elson, L. A., 605, 651
 Elson, R., 626, 658
 Elster, S. K., 395, 414, 444, 452, 456
 Elvehjem, C. A., 541, 543, 560, 562, 649, 654, 655, 669, 673, 674, 688, 690, 692, 693, 694
 Emanuel, S., 288, 289, 290, 295, 296, 306, 310, 332, 333
 Embden, G. M., 207, 217
 Emerson, G. A., 516, 519, 543, 555, 649, 683, 690

- Emerson, O. H., 543, 559, 683, 690
 Emery, W. B., 677, 689
 Emik, L. O., 270, 272, 277, 283
 Emmart, E. W., 432, 459
 Emmelin, N., 95, 96, 112
 Emmerson, K., Jr., 270, 283
 Engel, M. B., 451, 456
 Engel, R. W., 676, 690
 Engelbach, W., 626, 649
 Engle, M. C., 33, 53
 Engman, M. F., 314, 324, 333, 410, 414, 502, 506, 510, 511, 512, 513, 633, 648, 688, 694
 Engman, M. F., Jr., 688, 694
 Ensminger, M. E., 648
 Eppinger, H., 520, 555
 Epstein, A. L., 629, 649
 Epstein, N. N., 685, 690
 Eränkö, O., 575, 579
 Erisman, F., 239, 242
 Erlanger, J., 126, 127, 149, 176, 194
 Ernst, H. W., 246, 266
 Ernstone, A. C., 582, 583, 583
 Ershoff, B. H., 649
 Erway, W. F., 531, 555
 Eschenbrenner, A. B., 569, 578
 Euler, B. von, 649, 668, 690
 Euler, H. von, 649
 Euler, U. S. von, 86, 90, 112
 Evans, C. L., 72, 73, 101, 112
 Evans, D. G., 573, 578
 Evans, G. F., 632, 652
 Evans, H. M., 300, 305, 382, 388, 398, 413, 543, 555, 559, 683, 690
 Evans, R., 1, 7
 Evans, R. D., 67, 112
 Evans, V. J., 680, 686, 688, 696
 Evans, W. C., 524, 555
 Everett, M. R., 666, 673, 680, 688, 690
 Eyster, W. H., Jr., 80, 94, 112
 Facq, J., 345
 Fagerström, E., 627, 652
 Fahrig, C., 466, 472, 477, 480
 Fantes, K. H., 677, 689
 Fantl, P., 469, 481
 Farber, S., 634, 649
 Farmer, C. J., 534, 553
 Farmer, L., 98, 112
 Fasal, H., 619, 630, 649
 Favre, M., 38, 46, 53
 Feder, A., 676, 697
 Feinberg, S. M., 95, 97, 112
 Feindel, W. H., 133, 142, 152
 Feldberg, W., 95, 97, 112, 163, 174, 194, 453
 Fell, H. B., 382, 385, 386, 387, 388, 389, 394, 413, 414, 443, 455, 456, 575, 578
 Fellows, J. K., 49, 56
 Felsher, Z., 156, 184, 194, 234, 235, 236, 237, 239, 242, 261, 267, 377, 389, 405, 406, 414, 522, 549, 552, 555, 561, 699, 700, 701, 702, 706
 Ferguson, J. H., 381, 387, 547, 548, 554
 Ferguson, R. L., 30, 41, 54
 Ferguson, S., 46, 54
 Ferguson, W. S., 534, 555
 Fernholz, E., 684, 689
 Ferreira-Marques, J., 586, 599
 Ferris, E. B., 75, 112
 Festenstein, G. N., 329, 330, 333
 Fidler, E., 96, 117
 Field, J. B., 684, 695
 Fierst, S. M., 75, 101, 108
 Fieser, L. F., 322, 323, 324, 333
 Fieser, M., 322, 324, 333
 Figge, F. H. J., 527, 548, 555, 673, 694
 Filehne, W., 175, 194
 Finch, C. A., 216, 218
 Finesinger, J. E., 10, 19, 23
 Finkelstein, J., 665, 690
 Finkle, J. R., 208, 220
 Finkle, R., 208, 220
 Finkler, B., 58
 Finkler, R. S., 626, 649
 Finley, C. B., 130, 149
 Finnerud, C. W., 685, 690
 Fiocco, G. B., 633, 649
 Fiola, S., 540, 561
 Fischel, E. E., 452, 456
 Fischer, A., 385, 388, 447, 456
 Fischer, E., 337, 342, 362
 Fischer, R. B., 522, 559
 Fishberg, E. H., 202, 210, 211, 217, 222, 228, 229, 230
 Fisher, A. A., 534, 555
 Fisher, C., 150, 174, 198
 Fisher, I., 575, 578
 Fisher, J. K., 318
 Fitzpatrick, T. B., 520, 522, 523, 524, 528, 529, 530, 531, 532, 533, 534, 535, 536, 538, 542, 543, 544, 545, 546, 550, 551, 552, 553, 554, 555, 558, 562, 568, 573, 578, 665, 691
 Flataker, L., 442, 464
 Fleisch, A., 101, 112
 Fleischer, J. H., 46, 55
 Flemister, L. J., 496, 497, 511
 Flemming, W., 419, 456
 Flesch, P., 33, 41, 49, 54, 58, 352, 363, 370, 371, 372, 385, 388, 389, 518, 532, 555, 561, 584, 599, 611, 630, 633, 634, 649, 650, 668, 695, 699, 704, 706
 Flexner, L. B., 64, 111
 Flochs, K., 75, 101, 108
 Florey, H. W., 76, 117
 Flury, F., 45, 54
 Flynn, R., 625, 650
 Foerster, O., 89, 112, 136, 149, 175, 178, 190, 194
 Foged, J., 253, 267
 Folin, O., 467, 469, 470, 480
 Folk, B. P., 500, 501, 511
 Folkers, K., 667, 670, 673, 677, 689, 692, 693, 695
 Follis, R. H., 650, 661
 Follis, R. H., Jr., 435, 456, 666, 667, 670, 671, 673, 674, 675, 683, 685, 687, 688, 691, 698
 Fonzés-Diacon, 642, 643, 650
 Foot, N. L., 409, 414
 Forbes, A. P., 300, 306, 640, 650
 Forbes, T. R., 548, 555
 Forman, L., 534, 535, 554
 Forssberg, A., 639, 650
 Forster, A., 606, 611, 650
 Forster, R., 245, 266
 Forster, W., 209, 219, 478, 481
 Foster, F. I., 79, 80, 102, 103, 106, 111, 117, 630, 656
 Foster, M., 518, 555
 Fostvedt, G., 42, 56
 Fostvedt, G. A., 545, 555
 Fourt, L., 355, 363
 Fox, C. L., Jr., 48, 59, 494, 495, 498, 501, 502, 508, 511, 514
 Fox, E. L., 603, 646
 Fox, M., 357, 361
 Foy, J. R., 685, 697
 Fraenkel, L., 627, 650
 Franchi, C. M., 410, 412, 414
 Franke, F. E., 104, 112, 157, 189, 194
 Frankel, S., 341, 342, 347, 348, 364, 574, 579
 Franklin, H. C., 597, 599
 Frantz, V. K., 439, 464
 Fraser, F. R., 101, 110
 Fraser, R., 300, 306, 625, 626, 628, 629, 645
 Frazier, C. N., 382, 388, 543, 560, 679, 680, 681, 691
 Freed, S. C., 103, 112
 Freeman, G. L., 19, 23
 Freeman, L. W., 522, 559
 Freeman, M. E., 430, 452, 456
 Freeman, M. V., 40, 54
 Freeman, N. E., 86, 100, 112, 116
 Freiman, D. G., 103, 116
 French, J. E., 435, 436, 437, 443, 454, 456, 457
 Freny, M. R., 316, 326, 333
 Frerichs, J. B., 704, 706
 Freud, J., 614, 650
 Freudenthal, W., 597, 599
 Frey, J. R., 382, 383, 389
 Frey, L., 179, 194
 Frey, M. von, 121, 123, 136, 137, 138, 142, 145, 146, 147, 149
 Fricke, H. H., 677, 691
 Fridrichsons, J., 327, 332
 Frieboes, W., 235, 242, 345, 362, 511, 590, 590
 Frieden, E. H., 544, 555
 Friedenthal, H., 615, 650
 Friou, G. J., 432, 457
 Fritz, I., 103, 115, 445, 459
 Fritz, J. C., 542, 555
 Fritzsche, H., 683, 693
 Fromm, H. J., 368
 Frommel, E., 38, 46, 53
 Frost, D. V., 541, 543, 556, 677, 691
 Fruton, J. S., 447, 457, 704, 706
 Fuchs, H., 618, 619, 650
 Fürst, K., 221, 230

- Fürth, O. von, 523, 556
 Fuhrman, F. A., 35, 55
 Fuhs, H., 616, 617, 618, 619, 650
 Fulton, J. F., 174, 195
 Fulton, J. K., 430, 457
 Funk, C., 665, 691
- Gad-Andresen, K. L., 203, 208, 217
 Gaddum, J. H., 90, 99, 109, 111
 Gafford, J. A., 630, 646
 Gagne, A. P., 248, 249, 250, 259, 260, 267, 269
 Gale, J. C., 407, 409, 414
 Galewsky, E., 619, 650
 Gamble, J. L., 500, 502, 511
 Ganguly, H. D., 644, 645, 645
 Gans, O., 50, 55, 375, 387, 503, 506, 507, 508, 511
 Gardell, S., 458
 Gardner, L. I., 641, 660, 661
 Garn, S. M., 625, 650
 Garratt, D. C., 326, 332
 Garrod, M., 363, 404, 405, 406, 407, 410, 411, 412, 415
 Garzoli, R. F., 429, 459
 Gasser, H. S., 126, 127, 131, 148, 149, 176, 194
 Gaté, J., 96, 99, 112
 Gaudin, O., 41, 55
 Gaul, L. E., 379, 380, 388
 Gavin, G., 676, 691
 Gaylor, J. B., 179, 199
 Geary, J. R., Jr., 612, 614, 635, 650
 Geay, F., 284, 286, 307
 Geets, W., 15, 23
 Geiger, E., 664, 691
 Geiger, W. B., 351, 352, 356, 362, 363
 Geiling, E. M. K., 102, 113, 544, 556
 Gellis, S., 298, 308
 Georgesco, M., 630, 648
 Gerbrands, R., 18, 23
 Gerbstadt, H., 12, 16, 23
 Geréb, S., 498, 511
 Gerking, S. D., 213, 217, 248, 260, 265, 267, 268, 282, 282
 Gerschler, H., 5
 Gersh, I., 397, 414, 433, 437, 440, 442, 447, 450, 452, 457, 697, 701, 706
 Gershberg, H., 612, 650
 Gerstl, B., 543, 559
 Gerstner, H., 12, 15, 16, 17, 23
 Geschwind, I. I., 544, 556
 Gessard, C., 523, 556
 Ghadially, F. N., 612, 613, 660
 Giachetti, A., 634, 650
 Gibbs, H. F., 614, 650
 Gibbs, O. S., 42, 43, 54
 Gibson, D. M., 430, 432, 464
 Gibson, T. E., 160, 194
 Giddings, G., 466, 480, 483, 484, 490, 494, 496, 499, 511
 Gierke, E. von, 468, 480
 Gilbert, G. A., 355, 362
- Gildemeister, M., 15, 16, 18, 20, 23
 Gilding, H. P., 71, 73, 105, 117
 Gilje, O., 65, 68, 69, 112, 113
 Gillespie, D. T. C., 314, 333
 Gillespie, J. M., 372, 388, 643, 649
 Gilliat, R. W., 87, 112
 Gilligan, D. R., 395, 410, 415
 Gillman, J., 542, 556
 Gillman, T., 542, 556
 Gilman, A., 52, 55, 101, 114, 169, 194
 Gilmer, B. von H., 145, 149
 Ginsberg, B., 518, 532, 556
 Ginsberg, D., 295, 296, 307, 319, 333
 Ginsberg, J. E., 685, 691
 Ginsburg, D., 318, 335
 Giroud, A., 344, 352, 360, 362, 367, 370, 372, 388
 Gittes, H. R., 46, 55
 Glanzmann, E., 624, 650
 Glaser, E. M., 106, 107, 108, 113
 Glass, S. J., 640, 650
 Glendy, R. E., 102, 113
 Glick, D., 429, 432, 457, 575, 578, 685, 690
 Glücksmann, A., 587
 Glynn, L. E., 450, 452, 457
 Goadby, H. K., 18, 23
 Goadby, K. W., 18, 23
 Goddard, D. R., 356, 362
 Goebel, W. F., 422, 452, 458
 Goehl, R., 220
 Göpfert, H., 104, 113
 Goepp, R. M., 422, 461
 Goetz, R. H., 88, 105, 110, 113
 Goggio, A. F., 87, 111
 Gohlke, 621, 650
 Gold, N. I., 394, 414
 Goldberger, J., 668, 691, 697
 Goldbloom, A., 665, 694
 Goldblum, R. W., 650, 653, 685, 690
 Goldfarb, A. R., 543, 559
 Goldfarb, L., 288, 308
 Goldinger, J. M., 472, 479
 Goldman, L., 47, 55, 542, 560, 624, 647, 650
 Goldscheider, A., 133, 149
 Goldschmidt, S., 45, 54, 55
 Goldsmith, W. N., 550, 556
 Goldstein, M. S., 103, 115, 445, 459
 Goldstone, S. B., 33, 54, 385, 633, 649, 699, 704, 706
 Goldwasser, E., 52, 59
 Goldzieher, J. W., 4, 7, 517, 556
 Goldzieher, M. A., 4, 7, 517, 556
 Golla, F. L., 20, 23
 Golodetz, L., 310, 324, 336, 367, 372, 390, 486, 491
 Golovanoff, M., 44, 55
 Gomori, G., 331, 333, 435, 436, 457, 575, 576, 578
 Gompertz, M. L., 623, 660, 676, 697
 Gonell, H. W., 399, 414
- Goodell, H., 120, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 148, 149, 150, 151, 152
 Goodman, H., 633, 650
 Goodman, J. I., 270, 283
 Goodman, L. S., 169, 194
 Goodyear, G. H., 672, 697
 Gordon, E. S., 669, 691
 Gordon, H. H., 665, 693
 Gordon, M., 536, 556, 599
 Gorin, M. H., 33, 51, 53
 Gorrie, D. R., 679, 691
 Gorter, F. J., 541, 556
 Gottschalk, H. R., 489, 491
 Gougerot, L., 16, 17, 23
 Gould, B. S., 394, 395, 414, 415
 Gould, R. G., 577, 578
 Graaf, H. J. de, 298, 300, 301, 302, 306
 Graciansky, P. de, 545, 560
 Graef, I., 546, 561
 Graham, C. E., 411, 414
 Graham, D. P., 305, 308
 Graham, D. T., 131, 132, 133, 136, 137, 138, 139, 141, 142, 149
 Grais, M. L., 429, 457, 572, 578
 Grand, C. G., 538, 556
 Granick, S., 434, 460
 Granroth, T., 96, 113
 Grant, R., 624, 650
 Grant, R. T., 75, 78, 87, 88, 89, 91, 92, 97, 101, 113, 115, 576, 578
 Grassheim, K., 24
 Grassman, W., 396, 414
 Graubard, M., 548, 556
 Gray, M., 344, 362
 Graybiel, A., 102, 113
 Green, H. G., 84, 109
 Greenberg, D. M., 681, 686, 688, 689, 691, 697
 Greenberg, S. S., 103, 116
 Greenblatt, R. B., 625, 628, 651
 Greene, C. R., 533, 563
 Greene, R., 641, 651
 Greene, R. R., 47, 57
 Greenstein, J. P., 348, 362, 539, 540, 556, 569, 578
 Greig, D. M., 186, 194
 Grekin, R. H., 681, 690
 Gricouroff, G., 634, 635, 651
 Griesemer, R. D., 475
 Griffith, A. S., 382, 388
 Griffith, F. R., 88, 118
 Griffon, H., 643, 651
 Grigory-Bratu, I., 622, 651
 Grimm, N. Y., 351, 364
 Grimson, K. S., 86, 105, 113
 Groedel, F. M., 45, 55
 Grokoest, A. W., 612, 647
 Grollman, A., 102, 113
 Gronhovd, G., 220
 Groody, M. E., 543, 556
 Groody, T. C., 543, 556
 Gross, B. A., 227, 231
 Gross, F., 104, 113

- Gross, J., 397, 407, 408, 409, 410, 411, 412, 413, 414, 422, 427, 438, 447, 450, 457, 458
 Gross, P., 479, 480, 542, 556, 633, 651, 679, 685, 688, 691
 Gross, R., 372, 374, 387
 Grossenbacher, H., 43, 55
 Grossman, M. I., 86, 90, 114, 140, 151, 166, 167, 168, 171, 194, 195, 199, 624, 650
 Groth, W., 154, 194
 Grow, M. H., 67, 110
 Grünbaum, A., 210, 219
 Grüssner, A., 677, 695
 Grundeberg, T., 534, 556
 Grupper, C., 535, 556
 Grushko, Y. M., 651
 Grzycki, S., 344, 362
 Gschaidner, B., 326, 336
 Gsell, J. L., 624, 651
 Gualdi, A., 190, 194, 467, 468, 479, 480
 Gubner, R., 634, 651, 677, 691
 Günthard, H. H., 336
 Guenther, E., 326, 333
 Guirard, B. M., 672, 693
 Gumpert, M., 631, 647
 Gunning, R. E. L., 102, 114
 Gunter, G. S., 431, 457
 Gunther, G., 36, 55
 Gunther, L., 641, 642, 643, 645
 Gustavson, K. H., 394, 397, 404, 405, 414, 416
 Guszman, J., 611, 651
 Gutmann, E., 123, 152
 Guttman, L., 87, 112, 123, 152, 173, 194
 Guyon, L., 410, 416, 447, 461
 György, P., 543, 556, 651, 663, 670, 673, 675, 689, 690, 691, 692, 693
 Haagen-Smit, A. J., 670, 693
 Haas, E., 432, 457
 Haber, A., 444, 463
 Haberman, R., 545, 550, 556, 557
 Haddow, A., 605, 651
 Haden, R. L., 536, 557
 Haefele, J. W., 325
 Haegel, L., 501, 513
 Häggquist, G., 591, 599
 Haeussler, H., 580, 583
 Hagedorn, H. C., 469, 480
 Hagen, E., 103, 113, 125
 Hagen, W., 62
 Haggard, E. A., 18, 23
 Hahn, F., 530, 557
 Hahn, L., 431, 457
 Hahn, P. F., 216, 217
 Haimovici, H., 166, 167, 168, 194
 Haines, B. M., 436, 456
 Hakansen, E. Y., 429, 457
 Halberg, R. J., 568, 578, 677, 693
 Haldane, J. S., 35, 59, 201, 202, 212, 213, 215, 217, 234, 235, 238, 242, 243
 Haldi, J., 466, 480, 483, 484, 490, 492, 494, 496, 499, 511, 514
 Hale, C. W., 431, 434, 435, 457, 460, 611, 614, 651
 Hall, C. E., 401, 407, 416
 Hall, D. A., 422, 438, 448, 450, 457, 463
 Hall, F. G., 202, 203, 210, 211, 215, 217, 222, 230
 Hall, T. C., 517, 544, 557
 Halliburton, W. D., 449, 457
 Halliday, E. G., 679, 693
 Halpern, B. N., 41, 55
 Halpin, J. L., 542, 555
 Ham, A. W., 584, 599
 Hamblen, E. C., 625, 640, 647, 651
 Hamilton, B., 501, 512
 Hamilton, G. T. C., 87, 109
 Hamilton, H., 439, 464
 Hamilton, H. L., 546, 549, 557
 Hamilton, J. B., 103, 111, 117, 186, 191, 197, 298, 302, 306, 330, 331, 334, 373, 389, 465, 480, 516, 519, 547, 549, 555, 557, 575, 576, 578, 613, 615, 616, 617, 625, 626, 629, 630, 631, 632, 651, 654, 655, 656
 Hamilton, J. G., 30, 31, 38, 39, 40, 53
 Hamilton, T. S., 186, 197, 211, 212, 215, 216, 217, 218, 219, 623, 658, 664, 696
 Hamilton, W. F., 498, 512
 Hanawa, S., 468, 480
 Hancock, W., 35, 59, 201, 202, 212, 213, 215, 217, 234, 235, 238, 242, 243
 Handler, P., 669, 676, 690, 692
 Hanelin, J., 633, 656
 Hanna, B. L., 518, 557
 Hannay, P. W., 450, 461, 703, 706
 Hansen, A. E., 685, 692
 Hansmann, G. H., 42, 43, 55
 Hanson, J., 387, 388, 584, 599
 Hardesty, M., 441, 457
 Hardt, L. L., 222, 230
 Hardy, J. D., 120, 123, 131, 132, 134, 135, 137, 138, 140, 142, 143, 149, 150, 151, 152, 160, 194, 238, 242, 246, 249, 252, 257, 267
 Hardy, M. H., 622, 651
 Hardy, W. G., 20, 23
 Harkins, M. J., 622, 661
 Harley-Mason, J., 526, 554
 Harmer, I. M., 90, 113
 Harpman, J. A., 123, 152
 Harpuder, K., 87, 118
 Harpur, E. R., 665, 694
 Harris, A., 550, 557
 Harris, H., 626, 651
 Harris, J., 103, 113
 Harris, K. E., 90, 113
 Harris, L. J., 382, 388, 670, 689
 Harris, M., 355, 356, 358, 362, 363
 Harris, R. S., 35, 59
 Harris, S., 543, 557
 Harris, S., Jr., 543, 557
 Harris, S. A., 670, 673, 692
 Harrison, F., 174, 197
 Harrison, H. E., 498, 500, 502, 512
 Harrison, I., 131, 135, 148
 Harrison, R. G., 680
 Harry, R. G., 42
 Hart, B. B., 541, 562, 651
 Hart, E. B., 649, 688, 692, 697
 Hart, E. R., 46, 55
 Hart, W. M., 531, 534, 560
 Hartwell, G. A., 542, 557, 651
 Harvalik, Z., 651
 Harvey, A. M., 101, 113
 Harvey, C. C., 666, 692
 Haskin, D., 297, 298, 299, 300, 302, 306
 Hass, G. M., 443, 451, 457, 458
 Hasselbalch, K. A., 107, 113
 Hastings, A. B., 495, 513
 Haugen, C., 215, 220
 Haugen, C. O., 202, 215, 216, 217, 219, 220
 Haurowitz, F., 337, 342
 Hauser, E. A., 451, 455
 Hauser, F., 314, 333, 363
 Hausknecht, W., 223, 231
 Hawk, P. B., 217
 Hawkins, V., 677, 697
 Hawkins, V. R., 670, 695
 Haxthausen, H., 227, 230, 541, 557
 Haxton, H. A., 179, 194
 Hayek, F. A., 120, 150
 Hayes, M. A., 430, 458, 572, 578
 Haynes, F. W., 104, 119
 Haythorn, S. R., 145, 149
 Hayward, J. C., 684, 693
 Hayward, W. G., 630, 651
 Hazel, O. G., 97, 114
 Hazen, H. H., 639, 651
 Head, H., 136, 150
 Heard, E. V., 651, 664, 692
 Heatley, N. G., 569, 577
 Hechter, O., 103, 113, 432, 433, 458
 Hediger, S., 34, 45, 55, 580, 583
 Heemeyer, R., 213, 217, 490
 Hegsted, D. M., 216, 218
 Hehre, E. J., 428, 458
 Heidelberger, C., 669, 692
 Heidelberger, M., 422, 423, 424, 452, 458, 669, 692
 Heilbron, I. M., 328, 333
 Heilbrun, L. V., 503, 512
 Heilmeyer, L., 451, 458
 Heiman, W., 328, 336
 Heimberger, H., 79, 113
 Heinbecker, P., 126, 148
 Helfenstein, A., 328, 679, 693
 Hellauer, H., 94, 118
 Hellauer, H. F., 90, 113, 118, 143, 152
 Heller, H., 238, 242
 Hellerstein, H. K., 532, 557
 Hellinga, G., 606, 652
 Hellman, L. M., 684, 694
 Helman, F. D., 329, 333
 Helmer, A. C., 329, 333

- Hemmeler, G., 44, 57
 Hempelmann, L. H., 639, 652
 Hendrix, J. P., 105, 113
 Hendry, E. B., 641, 642, 658
 Hendrych, F., 37, 48, 58
 Henriques, F. C., Jr., 252, 267, 268
 Henry, J. L., 593, 595, 597, 599
 Henschel, A. F., 265, 269
 Henschke, V., 552, 557
 Hensel, H., 259, 268
 Hentschel, H., 320, 333
 Herb, S. F., 681
 Heringa, G. C., 344, 354, 362, 367, 388
 Herrick, J. F., 108, 113
 Herrington, L. P., 248, 249, 250, 259, 260, 267, 269, 603, 652
 Herrmann, F., 29, 30, 32, 33, 34, 44, 47, 55, 56, 58, 154, 159, 160, 177, 194, 199, 221, 223, 225, 226, 230, 258, 268, 270, 272, 280, 281, 282, 283, 289, 291, 293, 294, 295, 305, 306, 308, 310, 333, 494, 503, 506, 508, 512
 Hertzman, A. B., 74, 75, 87, 104, 111, 112, 113, 157, 159, 189, 194, 198
 Herzenberg, H., 182, 195
 Herzog, R. O., 399, 414
 Hesbacher, E. N., 51, 58
 Hess, A. F., 329, 333, 681, 692
 Hess, W. C., 352, 363
 Hesselbach, M. L., 540, 561
 Hetényi, G., Jr., 162, 171, 176, 195
 Hettig, R. A., 216, 217
 Heuser, G. F., 543, 559
 Heuss, E., 227, 230
 Heymans, C., 104, 113
 van Heyningen, R., 202, 210, 211, 220, 268
 van Heyningen, W. E., 397, 413, 416, 433, 461
 Heyns, U., 352, 360
 Heyroth, F. F., 633, 652
 Hier, S. W., 204, 207, 217, 222, 230
 Highberger, J. H., 395, 396, 397, 407, 409, 414, 422, 447, 457, 458
 Hightower, D. P., 670, 696
 Hilditch, T. P., 315, 328, 332, 333
 Hill, L., 175, 195
 Hillegas, A. B., 318
 Hills, O. W., 666, 692
 Hines, E. A., 71, 109
 Hines, L. E., 444, 462
 Hinman, W. F., 679, 693
 Hinsey, J. C., 622, 652
 Hirst, E. L., 677, 692
 Hitschler, W. J., 111
 Hobby, G., 539
 Hobby, G. L., 429, 460
 Hobson, R. P., 397, 414
 Höber, R., 405, 414
 Högberg, B., 544, 557
 Hoepke, H., 154, 195, 375, 388, 584, 599
 Hörstadius, S., 538, 557
 Hoff, F., 618, 621, 622, 625, 652
 Hoff, H. E., 105, 114
 Hoffman, J. G., 595, 596, 599, 639, 652
 Hoffmann, J., 652
 Hofmeister, F., 337, 342, 363, 405, 414
 Hogan, A. G., 652
 Hogeboom, G. H., 523, 531, 557
 Hogrebe, H., 13, 25
 Holaday, D., 672, 697
 Holborow, E. J., 430, 449, 452, 455, 457, 458
 Holland, A., 667, 692
 Hollander, L., 632, 652
 Holling, H. E., 75, 88, 101, 113, 114
 Holmberg, C. G., 688, 692
 Holmer, A. J. M., 627, 652
 Holmgren, H. J., 613, 646
 Holmquest, H. J., 14, 24
 Holt, L. E., 670
 Holtschmidt, 621, 650
 Holyoke, J. B., 191, 193, 195
 Homma, H., 186, 195
 Hommes, M., 654
 Hooker, C. W., 301, 306, 398, 415, 549, 560, 666, 692
 Hooper, J. H., 542, 555
 Hopf, G., 216, 217, 222, 230
 Hopkins, J. G., 97, 114, 318, 611, 652
 Hopmann, F., 40, 55
 Horn, D. H. S., 315, 321, 333
 Horn, G., 154, 195
 Horn, M. J., 356, 363
 Horner, W. E., 180, 195
 Horrigan, D., 677, 697
 Horton, B. T., 95, 114, 253, 254, 255, 256, 267, 268, 269, 535, 560
 Horton, J. W., 15, 24
 Horváth, G., 509, 514, 704, 707
 Horvath, P. N., 172, 198, 378, 389
 Horvath, S. M., 248, 265, 267, 268, 272, 277, 278, 279, 280, 281, 282, 283
 Horwitt, M. K., 666, 669, 692
 Hoskins, R. G., 102, 114
 Hotchkiss, R. D., 435, 458
 Hou, H. C., 285, 306, 324, 333
 Houchin, O. B., 673, 695
 Hougen, F. W., 315, 321, 333
 Houssay, B. A., 300, 308, 544, 557
 Houssay, F., 626, 653
 Houston, M., 550, 561, 586, 598, 600
 Hove, E., 688, 692, 697
 Howard, M., 611, 646
 Howe, P. R., 382, 390, 680, 698
 Howes, E. L., 444, 446, 454, 462
 Hu, C. K., 382, 388, 680, 691
 Hubbard, D. M., 644, 658
 Hubbard, L. H., 670, 696
 Huber, W. H., 611, 652
 Hubert, G., 516, 519, 547, 555, 557
 Hubert, G. R., 103, 117, 630, 656
 Hudson, M. F., 270, 281, 282
 Hudson, R. F., 355, 361
 Huf, E. G., 35, 55
 Huff, J. W., 669, 695
 Huff, R. L., 639, 660
 Huff, S. E., 69, 114
 Huggins, M. L., 400, 404, 407, 415
 Hughes, J., 131, 148
 Hume, E. M., 318, 335, 484, 491
 Hummel, R., 323, 335, 491
 Humphrey, J. H., 424, 430, 431, 452, 458
 Humphreys, H., 673, 698
 Humphreys, S., 661, 666, 670, 689, 698
 Hundley, J. M., 541, 557, 672, 692
 Hunt, C. H., 666, 692
 Hunt, M., 633, 650
 Huntington, R., 451, 458
 Hurley, H. J., 168, 181, 182, 183, 184, 186, 187, 188, 199, 222, 232
 Hurley, N. A., 509, 512
 Hurxthal, L. M., 626, 629, 652
 Huss, F., 324, 325, 334
 Hyde, J. E., 549, 560
 Hyndman, O. R., 88, 114, 164, 165, 195
 Ichihashi, T., 33, 55
 Idler, D. R., 323, 333, 487, 490
 Ikebata, T., 477, 482
 Ikeuchi, K., 158, 159, 177, 195, 234, 236, 239, 242, 652
 Illig, L., 81, 104
 Incedayi, C. K., 325, 333
 Ing, R. B., 541, 557, 672, 692
 Ingle, D. J., 300, 305, 398, 413
 Ingraham, L. P., 670, 689
 Ingraham, R. C., 500, 501, 503, 504, 505, 510
 Ingram, P. W., 100, 114
 Ingram, W. R., 174, 198
 Iob, L. V., 685, 691
 Iono, Y., 374, 388
 Ipsen, J., 253, 268
 Irmes, J. R. M., 382, 388
 Isbell, H., 674, 696, 697
 Ishii, Y., 315, 333
 Isler, H., 41, 55
 Issekutz, B., Jr., 162, 171, 176, 195
 Istók, J., 304, 306
 Itallie, L. V., 652
 Ito, M., 549, 557
 Ivy, A. C., 245, 268
 Jacob, A., 629, 652
 Jacobi, O., 228, 230
 Jacobs, E. C., 629, 652
 Jacobs, J. L., 341, 342, 347, 363
 Jacobs, K., 670

- Jacobs, M. H., 29, 55
 Jacobson, V. C., 520, 557
 Jacquet, L., 139, 140, 141, 150
 Jadassohn, J., 3, 7, 99, 114
 Jadassohn, W., 46, 55, 547, 557
 Jaddou, J., 228, 231
 Jaffé, R., 611, 623, 652
 Jager, B. V., 52, 53
 Jahncke, W., 399, 414
 Jakus, M. A., 401, 407, 416
 Jalowy, B. O., 652
 Janes, J., 431, 448
 Janitzky, A., 45, 55
 Jankowsky, W., 518, 557
 Janowitz, H., 86, 90, 114, 140, 151, 160, 161, 165, 166, 167, 169, 171, 194, 195
 Jansen, C. H., 329, 333
 Jaques, L. B., 96, 117
 Jaques, R., 532, 557
 Jarrold, T., 677, 697
 Jausion, H., 623, 652
 Jeannerat, J., 405, 413
 Jeger, O., 326, 327, 335, 336
 Jeghers, H., 516, 545, 550, 551, 557
 Jeney, A. von, 443, 458
 Jenkins, W. L., 145, 150
 Jenrette, W. V., 539, 540, 556
 Jensen, H. L., 359, 363
 Jensen, N. B., 469, 480
 Jersild, T., 679, 692
 Jervis, G. A., 542, 557, 664, 692
 Jesionek, A., 107, 114, 519, 547, 557
 Jochims, J., 4, 7
 Jodar, E. O., 547, 557
 John, F., 189, 195, 303, 306, 621, 652
 Johnson, B., 265, 267
 Johnson, B. C., 211, 212, 217, 218
 Johnson, H. H., Jr., 669, 692
 Johnson, H. J., 104, 114
 Johnson, J. L., 634, 645
 Johnson, L. V., 666, 690
 Johnson, M. W., 265, 267
 Johnson, P. L., 613, 652
 Johnson, R. E., 211, 212, 213, 214, 218, 219, 265, 268
 Johnson, S., 544, 557
 Johnson, S. A. M., 351, 364
 Johnson, W., 219
 Johnston, G. W., 37, 56
 Jolliffe, N., 670, 692
 Jones, D. B., 356, 363
 Jones, E. S., 654, 673, 694
 Jones, H. B., 688, 696
 Jones, H. S., 213, 214, 218, 265, 268
 Jones, I. S., 445, 458
 Jones, K. K., 289, 291, 293, 294, 306, 307, 310, 333, 528, 563
 Jones, P. J., 670, 689
 Jones, R., Jr., 634, 659
 Jones, T. S. G., 316, 332
 Jonesco, G., 88, 115
 Jong, J. C. de, 208, 220, 228, 229, 232
 Jongh, S. E. de, 382, 388
 Jonkhoff, A. R., 613, 652
 Joplin, R. J., 633, 656
 Jores, A., 195, 233, 234, 236, 238, 239, 242
 Jorpes, J. E., 448, 458
 Josefson, A., 627, 652
 Joseph, M., 622, 652
 Joseph, W., 624, 652
 Joy, H. H., 531, 557
 Jürgensen, E., 156, 161, 195, 233, 238, 242
 Jukes, T. H., 667, 677, 689, 692, 695
 Juon, M., 624, 652
 Jurenka, W., 302, 306
 Juster, E., 550, 558
 Juster, M. E., 629, 652
 Kaczka, E. A., 667, 692, 693
 Kadanoff, D., 621, 653
 Kähler, H., 611, 624, 654
 Kahlenberg, L., 36, 37, 56
 Kahler, H., 538, 559, 611, 624, 654
 Kahn, D., 160, 164, 195
 Kamen, M. D., 216, 217, 490
 Kaminester, S., 102, 117
 Kamm, E. D., 328, 333
 Kanagy, J. R., 393, 395, 397, 404, 413, 415
 Kanof, A., 318
 Kaplan, M. H., 452, 455
 Kaplan, N. D., 672, 693
 Karitzky, B., 202, 211, 218, 222, 230
 Kark, R., 684, 693
 Karnofsky, D. A., 52, 53
 Karp, F. L., 221, 223, 226, 230
 Karpelis, E., 624, 653
 Karrenberg, C. L., 633, 653
 Karrer, P., 328, 679, 683, 693
 Kashiwabara, I., 239, 242
 Kass, E. H., 431, 458
 Kastle, J. H., 523, 557
 Katai, K., 621, 655
 Katersky, E. M., 395, 410, 415
 Kato, H., 175, 196
 Katsuki, D., 208, 220
 Katsuki, S. S., 208, 220
 Katuno, M., 321
 Katz, G., 97, 114
 Katzberg, A., 592, 593, 595, 596, 599
 Katzenstein, K. H., 87, 104, 108
 Kaunitz, H., 681
 Kausche, G. A., 530, 557
 Kawabe, M., 351, 363
 Kawahata, A., 158, 161, 195
 Kaye, M., 404, 406, 407, 409, 415, 701, 706
 Keech, M. K., 430, 449, 455, 458
 Keele, C. A., 143, 160, 164, 165, 166, 167, 168, 193
 Keil, H. L., 541, 558
 Keilin, D., 688, 693
 Keller, A. D., 174, 195
 Keller, P., 11, 12, 13, 19, 21, 24, 25
 Keller, R., 154, 190, 199, 270, 283
 Kelley, W. E., 270, 283
 Kelly, E., 674, 693
 Kemmerer, K. S., 368, 390
 Kendall, F. E., 423, 424, 452, 458
 Kenedy, D., 71, 115
 Kennard, M. A., 174, 195
 Kennedy, F. A., 621, 653
 Kennedy, J. L., 9, 24
 Kensler, C. J., 666, 696
 Kenten, R. H., 394, 395, 396, 397, 409, 411, 412, 413
 Kepecs, J. G., 305, 307
 Kepler, E. J., 626, 628, 629, 653
 Kerb, J., 493, 494, 510
 Keresztesy, J. C., 543, 555, 649, 670, 693, 696
 Kertesz, D., 530, 558
 Kesler, R. L., 685, 690
 Kesten, B. M., 97, 114
 Keston, A. S., 498, 508, 511
 Keuning, F. J., 706
 Key, J. M., 632, 659
 Keys, A., 4, 7, 69, 104, 114, 212, 218, 265, 269, 558
 Kierland, R. R., 80, 94, 112
 Kile, R. L., 325
 Killough, J. H., 429, 459
 Kimura, G., 44, 56
 Kincaid, R. K., 213, 219, 265, 268
 King, C. G., 427, 460, 677, 693
 King, L. S., 706
 Kinney, T. D., 216, 218
 Kirby-Smith, H. T., 111
 Kirchner, E., 577
 Kiriluk, L. B., 429, 457
 Kirk, E., 3, 7, 100, 114, 296, 297, 307, 324, 325, 333
 Kirk, J. E., 3, 8
 Kirkwood, S., 648, 676
 Kirschbaum, A., 549, 560
 Kirson, M., 486, 491
 Kisin, E. E., 167, 195
 Kittsteiner, C., 213, 214, 218, 222, 231
 Klaar, J., 183, 196
 Kläwi, K., 324, 334
 Klauder, J. V., 227, 231, 503, 504, 509, 512, 623, 646, 686, 693
 Klein, A. E., 304, 307
 Klein, R., 641, 661
 Klein, W., 204, 219
 Kleipool, R. J. C., 634, 653
 Kleitman, N., 19
 Klemperer, P., 444, 450, 458
 Kligman, A. M., 295, 296, 307, 319, 333, 446, 454, 584, 599, 605, 613, 635, 639, 653, 699, 706
 Klinge, F., 302, 307
 Klopstock, E., 472, 477, 482
 Kneller, M. H., 421, 459
 Knisely, M. H., 66, 102, 114
 Knoche, H., 125
 Knowles, F. C., 619, 653
 Knowlton, K., 664, 695
 Knowlton, N. P., Jr., 587, 595, 596, 599
 Knudson, A., 485, 486, 491

- Kobrin, H., 166, 167, 168, 199
 Koch, E., 611, 653
 Koch, E. M., 329, 333
 Koch, F. C., 329, 333
 Kodicek, E., 394, 413, 443, 455
 Koelle, G. B., 101, 114, 186
 Kölliker, A., 446, 459
 Koenig, R., 210, 217, 478, 479, 490
 Königsbauer, H., 535, 558, 633, 653
 Königstein, H., 140, 143, 144, 499, 512, 550, 558, 612, 653
 Koets, P., 640, 653
 Kogoj, F., 618, 653
 Kohler, R., 94, 114
 Kokas, E. von, 660
 Kolb, L. C., 674, 696
 Kolnitz, H. von, 108, 114
 Kolpak, H., 412, 415
 Koniuszy, F. R., 677, 695
 Kooyman, D. J., 314, 324, 333, 483, 484, 485, 486, 487, 488, 489, 490, 506, 507, 512
 Koppányi, T., 168, 174, 196, 199
 Koppenhoeffer, R. M., 490, 490
 Kopsch, Fr., 350, 363
 Korkisch, H., 223, 231
 Kornberg, A., 670, 684, 693
 Kosaka, T., 177, 196
 Koskowski, W., 108, 114
 Kostermans, D., 634, 653
 Kovacs, J., 101, 114
 Kovacs, R., 50, 56, 101, 114
 Kozam, G., 613, 653
 Krafka, J., Jr., 681, 693
 Kramer, K., 32, 45, 56, 262, 268, 580, 582, 583
 Krampitz, L. O., 677, 698
 Kratky, O., 400, 415
 Krause, 158, 196
 Kraut, H., 644, 653
 Kredel, F. E., 173, 196
 Krehl, W. A., 669, 693
 Kreidl, A., 239, 242
 Kremen, A. J., 429, 457
 Krichevskaya, E. D., 666, 694
 Krider, M. M., 655
 Kringstad, H., 543, 558, 559
 Kröner, W., 187, 196
 Krogh, A., 61, 63, 65, 66, 67, 76, 79, 88, 102, 114, 582, 583
 Krohn, L., 103, 113
 Kromayer, E., 590, 599
 Krüger, L., 518, 558
 Krugh, F. J., 632, 652
 Krumdieck, N., 451, 458
 Kruse, H. D., 686, 693, 694
 Krysa, H. F., 532, 561
 Kubowitz, F., 530, 558
 Kudo, K., 182, 196
 Kuehl, F. A., Jr., 677, 689
 Küstermann, W., 154, 196
 Kugelmass, I. N., 679, 693
 Kuhn, R., 665, 693
 Kuhn, W., 621, 660
 Kulchar, G. V., 139, 151, 469, 478, 480, 481
 Kummerow, F. A., 656
 Kung, S. K., 331, 333, 576, 578
 Kunkel, P., 75, 101, 102, 114, 118
 Kuno, Y., 19, 20, 24, 156, 157, 158, 159, 160, 161, 162, 164, 165, 166, 168, 176, 177, 180, 181, 182, 183, 184, 188, 189, 195, 196, 234, 236, 238, 239, 242, 258, 268
 Kuntz, R. E., 429, 459
 Kunz, K., 201, 219
 Kunze, F. M., 31, 43, 48, 49, 51, 52, 56
 Kuo, K. W., 177, 182, 183, 196
 Kuo, N. H., 4, 8
 Kupel, C. W., 676, 688
 Kupperman, H. S., 549, 558
 Kuré, K., 175, 196
 Kurzer, F., 327, 332
 Kuwata, T., 315, 321, 333
 Kvorning, S. A., 3, 7, 295, 296, 307, 312, 314, 325, 333
 Kwiatowski, H., 90, 114
 Kylin, E., 625, 626, 653
 Kyrle, J., 262, 268
 Labesse, A., 546, 558
 Labout, J. W. A., 499, 511
 Lacey, O. L., 20, 23
 Lada, A., 527, 559
 Ladell, W. S. S., 201, 205, 206, 213, 214, 215, 218, 248, 265, 268, 270, 281
 Laden, E. L., 319, 320, 344, 363
 Laidlaw, P. P., 97, 111
 Lambert, E., 96, 115
 Landau, R., 629
 Landgrebe, F. W., 544, 563
 Landis, E. M., 64, 72, 73, 87, 115, 196, 459
 Landowne, M., 105, 115
 Landsteiner, K., 452, 459
 Langdon, R. G., 328, 333
 Langenskiöld, A., 179, 196
 Langer, K., 3, 8
 Langley, J. N., 89, 90, 93, 115, 166, 175, 183, 196
 Langworthy, O. R., 174, 196
 Lanius, B., 677, 691
 Lansing, A. I., 375, 376, 381, 388, 503, 504, 510, 512
 Lapidus, M., 677, 691
 Lapiere, S., 388
 Larnier, J., 467, 479
 Lasher, N., 297, 298, 300, 302, 304, 306
 Laskey, A. M., 432, 459
 Lassner, J., 618, 648
 László, D., 238, 242, 498, 511
 Latarjet, R., 522, 558
 Lattes, R., 444, 446, 454, 462
 Laug, E. P., 27, 31, 32, 34, 35, 38, 41, 43, 44, 45, 47, 48, 49, 50, 51, 52, 54, 56
 Lauritsen, M., 670, 689
 Lavietes, P. H., 203, 204, 218
 Lawrence, C. H., 302, 307
 Lawrence, E. O., 216, 217
 Lawrie, N. R., 331
 Layton, L. L., 443, 446, 459
 Lazar, M. P., 633, 659
 Lea, A. J., 520, 535, 546, 558
 Leach, E. A., 705, 706
 Leake, C. D., 568, 578
 Leary, W. V., 677, 689
 Lease, J. G., 674, 693
 Lebeuf, M. F., 624, 653
 Leblond, C. P., 360, 362, 372, 388, 593, 595, 596, 600
 Le Breton, R., 641, 642, 648
 Lederer, E., 314, 321, 323, 327, 329, 332, 333, 334
 Ledin, R. B., 318
 Le Double, A. F., 626, 653
 Leduc, E. H., 452, 455
 Leduc, S., 50, 56
 Lee, C. O., 37, 56
 Lee, T. H., 568, 578, 671, 693
 Lefébure, M., 621, 653
 Legg, J. W., 535, 558
 Lehmann, G., 36, 56
 Lehner, E., 71, 115
 Leiby, G. M., 344, 360
 Leifer, W., 318, 335
 Lemley, R. E., 644, 653
 Leninger, C. R., 174, 193
 Lennartz, E., 103, 115
 Lennert, K., 316, 321, 324, 325, 336
 Lennox, F. G., 405, 415
 Lens, J., 677, 697
 Lenstra, J. B., 208, 220, 228, 229, 232
 Lentz, J. W., 469, 470, 477, 481
 Léobardy, J. de, 546, 558
 Lepkovsky, S., 669, 670, 692, 693, 695
 Lepore, M. J., 494, 500, 509, 512
 Lepp, A., 543, 555
 Lerner, A. B., 476, 523, 524, 528, 529, 530, 531, 532, 533, 534, 535, 542, 543, 544, 545, 546, 552, 554, 555, 558, 568, 573, 578, 650, 653, 677, 685, 691, 693
 Leslie, A., 186, 193, 216, 216
 Leslie-Roberts, H., 53, 56
 Lespinne, V., 631, 653
 Letard, E., 604, 653
 Letterer, E., 459
 Leuthardt, F. M., 569, 578
 Levender, G., 590, 599
 Levene, P. A., 423, 459
 Leventhal, M. L., 626, 653, 658
 Lever, W. F., 377, 388, 509, 512, 514, 681, 693, 701, 706
 Levin, M. H., 186, 193, 216, 216
 Levin, O. L., 221, 222, 226, 231, 639, 653
 Levine, A., 434, 459
 Levine, M. D., 429, 459
 Levine, P., 459
 Levine, R., 103, 115, 445, 459
 Levine, S. Z., 242, 665, 693
 Lévy-Franckel, A., 550, 558
 Lewin, M. L., 540, 558
 Lewis, A. H., 534, 555
 Lewis, F. T., 180, 199

- Lewis, H. B., 621, 646, 651, 653, 664, 692
 Lewis, M. R., 599
 Lewis, R. A., 641, 661
 Lewis, T., 12, 21, 24, 61, 62, 67, 70, 71, 75, 79, 80, 82, 84, 88, 89, 90, 91, 92, 94, 95, 97, 98, 100, 105, 106, 115, 125, 127, 131, 142, 150, 170, 189, 196, 262, 263, 268
 Lewis, W. H., 599
 Li, C. H., 300, 305, 398, 413, 544, 556
 Li, H. C., 680, 691
 Lichstein, H. C., 673, 693
 Lichtman, S. S., 105, 109
 Lieber, G. D., 503, 512
 Liebert, E., 666, 692
 Liebow, A. A., 537, 558, 638, 639, 653
 Liese, G. J., 325, 336
 Liese, W., 247, 268
 Lietha, P., 45, 56
 Lifschütz, J., 315, 321, 323, 326, 332, 334
 Light, A. E., 630, 653
 Light, S. E., 535, 563
 Lightbody, H. D., 621, 653
 Lilienthal, J. L., Jr., 101, 113, 500, 501, 511
 Lillie, R. D., 432, 436, 437, 459
 Lillie, R. J., 543, 558
 Lincke, H., 222, 228, 229, 231, 310, 311, 314, 323, 324, 325, 334
 Linder, F., 178, 196
 Linderstrom-Lang, K., 359, 363
 Lindheimer, G. T., 679, 693
 Lindley, D. C., 648
 Lindley, H., 643, 649
 Lindner, E., 103, 112
 Link, K. P., 684, 695
 Linnell, L., 535, 558
 Linser, K., 611, 624, 654
 Linser, P., 321, 324, 334
 Lipmann, F., 672, 693
 Lipnik, M. J., 446, 454
 Lippincott, S. W., 654
 Lipson, M., 315, 334
 Lipton, M. A., 472, 479
 Lisco, H., 639, 652
 List, C. F., 164, 173, 174, 175, 178, 179, 180, 194, 196
 Litvac, A., 387, 387, 388
 Livingston, K. E., 134, 150
 Lloyd, D. J., 363, 392, 393, 404, 405, 406, 407, 409, 410, 411, 412, 415, 701, 706
 Lobitz, W. C., Jr., 81, 91, 94, 115, 154, 157, 158, 189, 190, 191, 192, 193, 195, 196, 201, 203, 204, 205, 209, 210, 212, 214, 218, 233, 242, 272, 279, 282, 479, 480, 510, 512
 Locke, E., 265, 268
 Locke, W., 213, 214, 218
 Lockhart, R. J. J., 534, 558
 Loeb, L., 381, 389
 Loebel, R. O., 569, 578
 Loeffler, R. K., 37, 56
 Löffler, W., 546, 553
 Löhner, L., 602, 654
 Loeschke, H., 182, 196
 Loewenthal, J. A., 534, 558
 Loewi, G., 450, 457
 Loewy, A., 235, 242
 Logan, M. A., 341, 342, 363, 395, 410, 416
 Lohmann, K., 665, 694
 Loman, J., 104, 115, 164, 169, 197
 Longo, V., 123, 138, 150
 Loofbourow, B. S., 395, 415
 Lord, J. W., Jr., 684, 688
 Lorente de Nó, R., 132, 150
 Lorenz, T. H., 305, 308
 Lorincz, A. L., 91, 115, 145, 150, 171, 173, 196, 304, 397, 416, 433, 437, 463, 531, 533, 535, 545, 552, 553, 558, 573, 579, 699, 706
 Lorus, J., 520, 562
 Louis, L. H., 265, 267
 Loula, L. C., 470, 479
 Love, L., 245, 266
 Lowe, J. S., 680
 Lowenstein, P. S., 627, 654
 Lower, E. S., 325
 Lowrey, G., 495, 512
 Lowry, E. I., 395, 414
 Lowry, O. H., 395, 410, 415
 Lu, T. W., 199
 Lubarsch, O., 468, 480
 Lucas, C. C., 353, 354, 361, 363
 Ludford, R. J., 488, 490
 Ludowieg, J., 428, 462
 Luduena, F. P., 49, 56
 Ludwig, A. W., 440, 441, 447, 454, 459
 Lüders, H., 321, 336
 Lueg, W., 24
 Lüneburg, 182, 197
 Lufkin, H. M., 123, 148
 Luithlen, F., 686, 694
 Lunde, G., 543, 558, 559
 Lurie, M. B., 441, 459
 Lusky, L. M., 633, 646
 Lussich Siri, J. J., 626, 628, 629, 655
 Lustig, B., 221, 226, 228, 231, 304, 307, 319, 320, 334, 335, 543, 559
 Lutz, R. E., 541, 559
 Lutz, W., 611, 654
 Lux, R. E., 35, 56
 Lyell, A., 620, 654
 Lyman, C. M., 472, 479, 672, 697
 Lynch, F. W., 410, 415
 Lynn, W. G., 545, 559
 Ma, C. K., 4, 8, 395, 415
 Macaulay, L. J., 134, 149
 McCall, K. B., 654, 665, 673, 674, 694, 697
 McCance, R. A., 681
 McCann, D. C., 680, 694
 MacCardle, R. C., 502, 506, 510, 511, 512, 513, 538, 559, 688, 694
 McCarthy, L., 287
 McCaughey, M., 643, 660
 McChance, H. A., 215, 218
 McClean, D. A., 428, 429, 430, 431, 432, 434, 449, 455, 460, 461
 McCleary, R. A., 10, 18, 20, 24, 173, 174, 177, 197
 McClellan, W. S., 32, 56
 McClure, W., 156, 158, 198
 McClure, W. B., 419, 461
 McCollister, D. D., 45, 53
 McCollum, E. V., 650, 654, 655, 665, 679, 680, 686, 688, 690, 691, 693, 694, 695
 McConahy, J. C., 51, 58
 McConnell, R. B., 669, 694
 McCracken, B. H., 517, 544, 557
 McCrea, J., 654
 McCreary, J. F., 666, 697
 McCulla, F. J., 630, 651
 MacDonald, A. D., 99, 111
 McDonald, F., 443, 458
 MacDonald, J. R., 431, 448, 458
 McDonald, N. D., 465, 479
 McElroy, L. W., 673, 694
 Macfarlane, R. G., 397, 415, 433, 461
 McGhie, J. F., 327, 332
 McGinnis, J., 543, 559
 McGlone, B., 45, 55, 123, 145, 148, 251, 266, 603, 654
 McGrath, L., 527, 553
 McHenry, E. W., 574, 577, 676, 691
 Macht, D. I., 39, 49, 56
 McIntire, F. C., 543, 556
 Macintosh, F. C., 96, 115
 McIntyre, J. M., 673, 690
 MacKee, G. M., 29, 30, 32, 33, 34, 47, 56, 58
 MacKenna, R. M. B., 305, 308, 312, 313, 321, 323, 324, 325, 327, 328, 329, 330, 334
 Mackie, G. C., 265, 269
 McKinley, W. A., 174, 193
 McLaughlin, G. D., 392, 393, 394, 405, 409, 415, 494, 495, 503, 513
 McLean, F. C., 504, 511
 MacLean, J. G., 509, 512
 MacLennan, J. D., 397, 415, 433, 461
 MacLeod, J. M. H., 410, 415
 McManus, J. F. A., 409, 415, 433, 435, 436, 437, 461
 McMaster, P. D., 420, 461
 McNally, W. D., 42, 56
 McQue, B., 359, 364
 McSwiney, B. A., 87, 111, 202, 218
 Madden, R. J., 669, 690
 Maddock, C. L., 464
 Madinaveitia, J., 431, 433, 459
 Maeda, S., 175, 196
 Magendantz, H., 238, 242

- Magoun, H. W., 133, 150, 174, 194, 197
 Mahli, W. H., 261, 268
 Major, R. T., 672, 697
 Malangeau, P., 42, 57
 Mallette, M. F., 530, 559
 Mallory, F. B., 410, 415
 Malméjac, J., 88, 115, 470, 480
 Malmgren, H., 434, 463
 Mancini, R. E., 447, 459, 572, 578
 Mandels, G. R., 359, 364
 Mander, J. V., 352, 363
 Mandur, J., 175, 198
 Manery, J. F., 495, 501, 513
 Mangieri, C., 449, 461
 Mangold, E., 363
 Mann, L., 16, 23
 Mann, T., 688, 693
 Manning, J. C., 630, 661
 Manz, E., 324, 325, 334
 Marchionini, A., 11, 25, 188, 197, 209, 210, 218, 221, 222, 223, 224, 226, 231, 324, 325, 334, 478, 480, 621, 639, 654
 Marcus, M. D., 704, 706
 Mardashev, S. R., 343, 344, 348, 353, 363, 574, 578
 Margot, A., 46, 55, 547, 557
 Marker, R. E., 326, 334
 Markert, C. L., 532, 559
 Markham, J. W., 143
 Marmelszadt, W. L., 679, 680, 681, 691
 Maroske, F., 518, 561
 Marples, E., 242, 665, 693
 Marrazzi, A. S., 46, 55
 Marriott, R. H., 397, 405, 406, 415
 Marschalko, T. von, 304, 307
 Marsh, D. F., 101, 115
 Marston, H. R., 352, 363, 688, 694
 Martin, A. J. P., 351, 354, 363
 Martin, D. W., 670, 696
 Martin, G. J., 96, 116, 534, 535, 543, 559, 654, 686, 694
 Martinotti, L., 375, 388, 389
 Marvin, H. M., 90, 94, 100, 170, 196
 Marzoni, F. A., 105, 113
 Maschmann, E., 397, 415, 433, 459, 573
 Maskilleysen, L. N., 666, 694
 Mason, H. L., 154, 157, 158, 189, 190, 196, 208, 218, 394, 417, 479, 480, 626, 628, 629, 653, 665, 697
 Mason, H. S., 520, 526, 527, 538, 554, 559
 Mason, L. M., 624, 650
 Masson, P., 79, 115, 538, 559
 Mathews, M. B., 425, 432, 459
 Mathieson, A. McL., 327, 332
 Matschak, H., 37, 56
 Matsumoto, Y., 467, 469, 470, 480
 Matthes, 107, 115
 Matthes, K., 104, 113
 Matthews, B. F., 453, 459
 Matthews, B. H. C., 89, 109
 Matthews, V. J., 484, 485, 488, 490
 Maximow, A. A., 446, 459, 460
 Maxwell, A. F., 630, 654
 May, C. D., 444, 463
 May, R., 634, 654
 Mayer, G. G., 58
 Mayer, R. L., 99, 115, 429, 460
 Mayr, J. K., 186, 197
 Mazur, M., 576, 578
 Mazza, F. P., 526, 559
 Medawar, P. B., 410, 416, 540, 553, 573, 578, 699, 706
 Meier, K., 624, 650
 Meirowsky, E., 521, 522, 559
 Meites, J., 622, 624, 654
 Meixner, M. D., 135, 137, 138, 143, 149, 150
 Melarango, H. P., 435, 460
 Melczer, N., 192, 197, 235, 242, 305, 307, 330, 331, 334
 Mellanby, E., 382, 385, 386, 387, 388
 Mellinkoff, J., 180, 197
 Mellinkoff, S. M., 180, 197
 Melton, F. M., 34, 42, 47, 49, 52, 53, 58, 140, 143, 150, 151
 Melville, D. B., 673, 690
 Memmesheimer, A., 154, 192, 199
 Memmesheimer, A. M., 69, 115
 Mendel, L. B., 679, 695
 Menkin, V., 100, 103, 115, 442, 460, 622, 647, 704, 706
 Mense, K., 619, 620, 654
 Mercer, E. H., 351, 363, 367, 374
 Mercier, O., 327, 334
 Merivale, W. H. H., 640, 641, 646, 654
 Merryman, M. P., 644, 653
 Mertens, V. E., 559
 Mescon, H., 190, 191, 192, 199, 352, 363, 371, 389, 468, 481, 634, 659
 du Mesnil de Rochemont, R., 5, 8
 Messer, A. C., 45, 58, 580, 581, 582, 583, 583
 Meyenberg, 40, 56
 Meyer, A., 485, 486, 490, 495, 513
 Meyer, H. H., 244, 268
 Meyer, J., 472, 473, 475, 476, 477, 479, 566, 568, 569, 570, 571, 577, 593, 595, 597, 599, 613, 646, 665, 689
 Meyer, K., 422, 423, 424, 425, 426, 428, 429, 430, 431, 432, 433, 434, 444, 445, 446, 448, 451, 452, 453, 454, 458, 460, 462, 572, 578
 Meyer, K. H., 391, 404, 405, 412, 413, 416
 Meyer-Lierheim, F., 604, 654
 Meyers, D. S., 49, 56
 Meyler, L., 654
 Michael, G., 632, 659
 Michael, M., 445, 460
 Michaelis, L. A., 356, 362, 434, 460
 Michelfelder, T., 58
 Mickelsen, O., 212, 218
 Miescher, G., 22, 24, 31, 49, 56, 277, 281, 282, 289, 290, 291, 293, 294, 298, 304, 377, 389, 522, 527, 552, 559
 Migeon, C. J., 641, 660, 661
 Mijoric, M. V., 327, 336
 Milberg, I. L., 106, 116
 Milbradt, W., 32, 37, 57, 675, 694
 Miles, A. A., 453
 Milhade, J., 397, 413, 573, 577
 Milhorat, A. T., 160, 194, 238, 242
 Miller, A. A., 679, 694
 Miller, C. G., Jr., 411, 412, 416
 Miller, C. S., 272, 282
 Miller, H., 475, 476, 480
 Miller, J. A., 670, 694
 Miller, L. B., 566, 568, 569, 570, 571, 577
 Miller, M. H., 670, 698
 Miller, M. L., 684, 694
 Miller, O. B., 37, 57, 58, 272, 282
 Miller, O. G., 699, 705
 Miller, T., 40, 54
 Miller, Z. B., 472, 473, 475, 476, 477, 479, 613, 646, 665, 689
 Milles, G., 103, 116
 Milne, M. J., 520, 562
 Mims, V., 676, 690
 Minard, D., 142, 150
 Minder, H., 522, 527, 552, 559
 Miner, D. L., 670, 694
 Mirskaia, L., 604, 648
 Miszurski, B., 385, 386, 389
 Mitchell, H. H., 186, 197, 211, 212, 215, 216, 217, 218, 219, 623, 658, 664, 696
 Mitchell, H. K., 676, 694
 Mixon, L. W., 326, 334
 Miyake, I., 471, 480, 583
 Mizell, L. R., 355, 356, 363
 Mobbs, R. F., 46, 57
 Moellendorff, W. von, 50, 54
 Moerner, C. T., 368, 389
 Moerner, K. A. H., 368, 389
 Mohammad, A., 543, 559
 Mohs, P., 323, 334, 490
 Mole, R. H., 250, 268
 Molinari Tosatti, P., 97, 118
 Molomut, N., 444, 445, 463
 Molony, C. J., 670
 Molt, F. H., 654
 Monacelli, M., 479, 480
 Moncorps, C., 29, 42, 45, 47, 57, 471, 480, 521, 559, 675, 694
 Montagna, W., 154, 181, 186, 191, 192, 197, 285, 288, 298, 306, 307, 330, 331, 334, 352, 373, 389, 435, 460, 465, 480, 575, 576, 578, 611, 613, 647, 654, 674, 694
 Montavon, M., 336
 Montgomery, D. W., 627, 654

- Montgomery, H., 71, 116, 192, 197, 235, 243, 520, 523, 532, 535, 536, 538, 542, 550, 551, 553, 555, 560, 568, 578
- Montgomery, M. L., 688, 694, 696
- Moog, O., 197, 233, 234, 239, 242
- Mook, W. H., 410, 414
- Moon, V. H., 445, 460
- Moore, D. D., 203, 204, 218
- Moore, H. P., 542, 555
- Moore, P. R., 324, 334, 490
- Moore, R. A., 684, 688, 689, 694
- Moore, R. C., 543, 556
- Moore, R. M., 622, 647
- Moore, T., 679, 694
- Morat, 106, 111
- Moretti, G. F., 545, 559
- Morf, R., 679, 693
- Morgan, A. F., 543, 559, 560, 666, 692
- Morgan, C. T., 145, 146, 150
- Morgan, J. K., 97
- Morgan, W. T. J., 452, 460
- Mori, S., 382, 389
- Moritz, R. A., 252, 267, 268
- Moritz, W., 382, 389
- Morrione, T. G., 448, 460
- Morris, H. P., 654
- Morris, J. E., 665, 694
- Morris, J. P., 654
- Morrissey, M. J., 639, 654
- Morton, A. A., 527, 560
- Morton, J. V., 444, 445, 463
- Morton, R. A., 329, 330, 333, 680
- Mosbach, E. H., 427, 460
- Moses, F., 428, 462
- Mosher, H. H., 208, 218
- Moskop, M., 381, 389
- Moss, J. A., 396, 413
- Moss, J. N., 96, 116
- Mottram, J. C., 613, 655
- Moult, F. H., 382, 389
- Mountain, J. T., 664
- Mouriquaud, G., 655
- Mowbray, R. R. de, 641, 646
- Moxon, A. L., 644, 655
- Mozingo, R., 673, 692
- Mu, J. W., 543, 560
- Müller, J., 284, 307
- Mueller, J. F., 677, 697
- Mueller, J. H., 368, 389
- Müller, L. R., 116
- Müller, O., 63, 64, 65, 66, 67, 69, 76, 92, 102, 106, 107, 116
- Muende, L., 410, 415
- Muhr, A. C., 326, 335
- Muir, W. R., 542, 560
- Mulinos, M. G., 104, 116
- Munch, J. C., 633, 655
- Mundy, W. L., 445, 455
- Murat, M., 95, 96, 99, 112, 116
- Murlin, J. R., 108, 110, 258, 259, 268
- Murphy, J. C., 318, 334
- Murray, J. F., 542, 553
- Murray, K. E., 321, 334
- Murray, M. R., 622, 655
- Murray, R. G. E., 430, 460
- Muschenheim, C., 246, 267
- Mussio-Fournier, J. C., 545, 560, 626, 627, 628, 629, 645, 655
- Muto, K., 166, 183, 197
- Myers, R. J., 615, 616, 617, 655
- Myerson, A., 104, 115, 164, 169, 197
- Nadel, A., 341, 342, 347, 363, 483, 490, 494, 495, 508, 509, 513
- Nadkarni, M. V., 49, 56
- Nagashima, T., 197
- Nageotte, J., 394, 410, 416, 447, 461
- Naide, M., 630, 655
- Nakagawa, T., 168, 171, 172, 199
- Nakamura, Y., 472, 482
- Nakanishi, K., 303, 307
- Narahara, K., 466, 467, 469, 471, 480
- Nathan, E., 494, 501, 503, 508, 509, 513
- Nathanson, A., 29, 57
- Naz, J. F., 633, 655
- Nealon, D. F., 531, 560
- Needham, D. M., 566, 569, 570, 571, 578, 704, 706
- Negelein, E., 477, 482
- Nelemans, T. G., 706
- Nelson, C. T., 681, 694
- Nelson, D., 47, 57
- Nelson, J. M., 528, 560
- Nelson, N., 248, 267
- Nelson, V. E., 541, 558
- Nenda, P., 627, 655
- Nesterow, 61, 116
- Netsky, M. G., 164, 197
- Neuberger, A., 394, 416, 550, 560
- Neuhaus, H., 229, 231
- Neuman, R. A., 341, 342, 363, 395, 396, 410, 411, 416
- Neumann, F. W., 655
- Neuroth, G., 249, 254, 268
- Neuwirth, I., 479
- Neville-Jones, D., 573, 578, 704, 706
- Newburgh, L. H., 233, 238, 243, 244, 246, 260, 268
- Newman, H. W., 74, 100, 119, 168, 172, 200
- Newman, L. H., 467, 469, 470, 480
- Newton, H. F., 622, 647
- Newton, J. K., 484, 485, 488, 490
- Newton, M., 245, 266
- Nexmand, P. H., 304, 307
- Nicholls, J., 543, 562, 658, 666, 670, 672, 673, 674, 696
- Nicholls, L., 542, 560
- Nichols, A. C., 188
- Nickerson, M., 101, 116, 518, 560
- Nicolaides, N., 296, 307, 312, 313, 314, 321, 325, 328, 329, 334
- Nicolaysen, R., 681, 694
- Nielsen, E., 670, 674, 693, 694
- Nielsen, M., 265, 268, 361
- Nielson, E., 543, 560, 655
- Nielson, P. E., 1, 7
- Niemann, C., 337, 340, 342
- Niles, H. D., 625, 655
- Nilzen, A., 95, 96, 113, 116
- Nishimura, H., 655
- Noback, C. R., 154, 181, 197, 285, 288, 307, 330, 331, 334
- Noble, G., 549, 560
- Norris, L. C., 543, 559
- Norrlind, R., 94, 116
- Nothacker, W. G., 444, 454
- Novak, L. J., 624, 655, 676, 694
- Novelli, G. D., 672, 693
- Novikoff, A. B., 394, 416, 575, 579
- Nowotny, H., 362
- Nutini, L. G., 569, 571, 577
- Nutting, G. C., 407, 416
- Nyström, A. E., 633, 655
- Oakley, C. L., 397, 416, 431, 433, 461
- Obermayer, M. E., 551, 553
- O'Brien, J. P., 270, 271, 272, 273, 274, 275, 280, 281, 282
- Ochs, M. J., 432, 457
- Odel, H. M., 37, 54, 535, 560
- Odland, G. F., 699, 706
- Oesch, F., 624, 655
- Oettel, H., 535, 560
- O'Flaherty, F. O., 409, 416
- Ogston, A. G., 426, 440, 453, 461
- Ohara, K., 164, 197, 581, 583
- Ohle, H., 316, 335
- Okajima, K., 288, 307
- Okamura, C., 621, 655
- Okinaka, S., 175, 196
- Okuda, S., 622, 655
- Okuda, Y., 621, 655
- Olcott, H. S., 680, 694
- Oldham, F. K., 544, 556
- O'Leary, J. L., 126, 148
- O'Leary, P. A., 65, 68, 69, 71, 116
- Oleson, J. J., 436, 462, 543, 560
- Oliver, E. A., 534, 535, 560, 562
- Oliver, J., 449, 461
- Olmanson, G., 219
- Onizawa, J., 486, 491
- Onslow, H., 523, 560
- van Oordt, G. J., 595, 599
- Opdyke, D. L., 375, 376, 381, 388
- Oppel, T. W., 123, 142, 149, 623, 655
- Oppenawer, R., 677, 695
- Oppenheim, M., 31, 57
- Oppenheimer, B. S., 104, 105, 109, 367, 372, 390
- Opsahl, J. C., 433, 441, 442, 461, 572, 579
- Ord, W. M., 438, 461
- Orekhovich, K. D., 409, 416, 444, 447, 450, 461
- Orekhovich, V. N., 409, 416, 447, 461
- Orent, E., 686, 693, 694
- Orent, E. R., 686, 694
- Orent-Keiles, E., 655
- Ormea, F., 189, 197
- Ormsby, O. S., 192, 197, 542, 560

- Oronty, L. R., 257, 268
 Orr, T. G., 536, 557
 Osborne, T. B., 679, 695
 Osbourne, R. A., 591, 599
 Oser, B. L., 217
 Osterberg, A. E., 218, 233, 242, 479, 480, 510, 512
 Ott, M. L., 431, 456
 Ottenstein, B., 161, 191, 197, 199, 209, 210, 211, 218, 220, 222, 231, 232, 324, 325, 333, 334, 467, 478, 480, 489, 491, 574, 579
 Ottenwaelder, A., 634, 655
 Overman, R. S., 684, 695
 Overton, E., 29, 57

 Pace, E. R., 685, 690
 Pack, G. T., 676, 688
 Paff, G. H., 449, 461, 632, 634, 655
 Page, A. C., Jr., 677, 695
 Page, R. M., 359, 573, 579
 Paglia, A., 632, 655
 Painter, E. P., 644, 655
 Pakheiser, T., 503, 511
 Palitz, L. L., 397, 416
 Palmer, A., 222, 230
 Palmer, J. W., 423, 460
 Palmer, W. W., 467, 481
 Palmes, E. D., 157, 193, 197, 238, 241, 243, 248, 265, 267
 Pana, C., 183, 197
 Pankhurst, K., 404, 416
 Pannattoni, M., 634, 656
 Parhon, C. I., 485, 491
 Park, C. R., 248, 265, 267
 Parker, F., Jr., 410, 415
 Parker, G. H., 549, 560
 Parker, L. F. J., 677, 696
 Parkurst, H. T., 372, 375, 376, 389
 Parmalee, A. H., 670
 Parmenter, H. S., 100, 109
 Parnas, J., 322, 335
 Parnell, J. P., 288, 306, 307, 324, 332, 612, 656
 Parrish, J., 35, 55
 Parrisius, 72, 116
 Parsons, H. T., 674, 693
 Parsons, R. J., 420, 461
 Partridge, S. M., 452, 460
 Paschkis, K. E., 531, 534, 560
 Paton, W. D. M., 96, 115, 172, 197
 Patt, H. M., 639, 656
 Patterson, W. I., 356, 363
 Pattle, R. E., 133, 150
 Patton, H. D., 166, 167, 197
 Patzelt, V., 375, 389, 584, 599
 Patzschke, W., 209, 219
 Paul, H. E., 488, 491
 Paul, M. F., 488, 491
 Paulais, R., 545, 560
 Pauling, L., 400, 401, 416
 Paulson, M., 661, 673, 698
 Pautrier, L. M., 699, 706
 Pavcek, P. L., 542, 560
 Peabody, R. B., 425, 426, 462
 Peacock, S. C., 664, 695
 Pearce, H., 666, 697
 Pearce, R. H., 423, 430, 440, 453, 460, 461, 464
 Pearson, R. S. B., 75, 87, 97, 101, 113, 576, 578
 Peck, S. M., 58, 318, 335, 535, 537, 540, 541, 558, 560, 611, 656
 Pedersen, A. L., 628, 640, 656
 Pedersen, J., 628, 630, 640, 656
 Peet, M. M., 164, 173, 174, 175, 179, 180, 196
 Peiser, B., 633, 647
 Pellerat, J., 95, 96, 99, 112, 116
 Pelletier, M. H., 101, 115
 Peña-Chavarria, A., 542, 560
 Penney, J. R., 443, 461
 Pennington, D., 673, 695
 Percival, G. H., 116, 450, 461, 703, 706
 Pereiro Miguens, M., 226, 231
 Perez, W. M., 681, 695
 Perl, E., 437, 461
 Perla, D., 103, 116
 Perlzweig, W. A., 669, 695
 Pernkopf, E., 375, 389
 Perrone, J. C., 394, 416
 Perutz, A., 42, 47, 57, 221, 226, 228, 231, 304, 307, 334, 335, 623, 656
 Pescatore, J. J., 44, 53
 Peschkowsky, P., 24
 Peters, A. D. K., 626, 656
 Peters, G. A., 626, 628, 629, 653
 Peters, J. P., 202, 203, 204, 218, 494, 499, 500, 513, 536, 560
 Peters, R. A., 472, 481, 569, 570, 571, 573, 577, 578, 579, 665, 695, 704, 705, 706
 Petersen, W. F., 103, 116, 704, 706
 Petit, A., 284, 286, 307
 Petrow, V. A., 326, 332
 Peukert, L., 223, 231
 Pfaltz, H., 656
 Pfeiffer, C. A., 301, 306, 398, 415, 549, 560, 666, 692
 Phemister, D. B., 173, 196
 Phillip, H., 357, 364
 Phillips, F. S., 52, 55
 Phillips, G., 85, 109
 Phillips, P. H., 648, 658, 676
 Philpot, F. J., 440, 461, 666, 695
 Philpot, J. St., L. 444, 461
 Pickering, G. W., 88, 115
 Pickett, W. J., 622, 646
 Pierce, J. V., 677, 689, 695
 Pigman, W. W., 422, 461
 Pike, R. M., 430, 461
 Pillat, A., 543, 560
 Pillsbury, D. M., 139, 151, 272, 277, 278, 281, 283, 318, 335, 446, 454, 466, 467, 469, 470, 472, 477, 478, 481
 Pimenta, A. de M., 178, 196
 Pincus, G., 548, 556
 Pinkus, F., 3, 8, 147, 150, 154, 155, 182, 197, 288, 307, 344, 364, 584, 589, 599, 607, 608, 615, 616, 619, 620, 621, 634, 639, 640, 656
 Pinkus, H., 193, 197, 586, 591, 592, 594, 599, 600, 611, 656
 Pinson, F. A., 233, 234, 243
 Pintus, G., 305, 307
 Piper, H. G., 228, 229, 231
 Piquet, J. C., 43, 57
 Pirani, C. L., 443, 444, 451, 461, 462
 Pirie, A., 666, 695
 Piriã, V., 575, 579
 Pischzek, F., 116
 Pisha, B. V., 154, 190, 199, 270, 283
 Pitts, G. C., 213, 214, 218, 265, 268
 Plas, G., 535, 556
 Plate, W. P., 656
 Platz, B. R., 670, 685, 695
 Platz, R. R., 656
 Pleass, W. B., 406, 415
 Plotinkova, N. E., 409, 416, 447, 461
 Plotz, C. M., 444, 446, 454, 462
 Pochin, E. E., 131, 150
 Pohl, J., 616, 656
 Poling, C. E., 543, 556
 Pollack, A. D., 450, 458
 Pollack, E., 626, 628, 629, 655
 Pollock, L. J., 133, 150
 Polunin, I., 573, 579
 Pond, H., 42, 43, 55
 Popper, A., 499, 513
 Popper, H., 330, 332
 Porges, N., 433, 462
 Porter, J. W., 518
 Porter, K. R., 394, 397, 416, 446, 447, 462, 463
 Pose, G., 41, 53
 Posener, K., 477, 482
 Potelunas, C. B., 135, 137, 138, 143, 149, 150
 Pozzo, A., 186, 197
 Pozzo, G., 12, 24
 Prail, P. F. G., 526, 554
 Prakken, J. R., 448, 462
 Pratt, A. W., 408, 410, 411, 416
 Pratt, E. L., 215, 218
 Prego, L. E., 41, 53
 Pritchard, J. E., 289, 308, 683, 689
 Prokoptschuk, A., 486, 491
 Prose, P. H., 159, 160, 177, 194, 225, 230, 258, 268, 270, 282, 289, 291, 293, 294, 295, 306, 310, 333, 429, 462
 Prunty, F. T. G., 444, 455
 Pugh, C. E. M., 523, 534, 560, 561
 Pulewka, P., 356, 364

 Quackenbush, F. W., 656, 670, 685, 695
 Quibell, T. H. H., 431, 433, 450
 Quinn, R. W., 432, 452, 462
 Quisenberry, J. H., 604, 656

- Raabe, S., 222, 230
 Rabbeno, A., 308
 Rabeau, H., 42, 57
 Raben, M. S., 544, 561
 Rabinowitch, I. M., 210, 220
 Rácz, S., 509, 514, 704, 707
 Rader, B., 245, 267
 Ragan, C., 433, 444, 445, 446, 454, 455, 462
 Ragni, G., 634, 656
 Rainone, S., 264, 268
 Rake, G., 344, 362
 Rakoff, A. E., 531, 534, 560
 Ralli, E. P., 546, 561, 562
 Ralph, P. H., 344, 364
 Ramage, H., 35, 36, 59, 239, 243
 Ramalingaswami, V., 681
 Rambacher, P., 356, 357, 364
 Ramey, E. R., 103, 115, 445, 459
 Randall, A., 664, 677, 689
 Randall, W. C., 156, 157, 158, 159, 161, 162, 165, 169, 176, 188, 189, 192, 194, 197, 198, 200, 233, 236, 238, 243
 Ranson, S. W., 85, 116, 128, 129, 150, 154, 174, 194, 197, 198
 Raoul, Y., 545, 560
 Raper, H. S., 523, 524, 525, 534, 535, 555, 558, 561
 Rappaport, F., 469, 481
 Rapport, M. M., 423, 425, 430, 431, 432, 434, 452, 453, 460
 Raschig, W., 45, 55
 Rask, O. S., 654
 Rasmussen, K. A., 51, 57
 Rauch, H., 611, 647, 674, 695
 Rauch, R., 535, 554
 van Ravenswaay, A. C., 15, 24
 Rawles, M. E., 538, 561, 586, 600
 Rawls, W. B., 517, 556
 Ray, C. T., 510
 Reardon, M. J., 105, 113
 Rease, P., 498, 510
 Rebell, C. G., 318
 Rebell, G., 318, 335
 Redisch, W., 264, 268
 Redlich, E., 628, 656
 Reed, L. J., 665
 Reed, R., 422, 438, 448, 450, 457, 463
 Reed, T. G., 430, 458
 Rees, A. L. G., 363
 Regelsberger, H., 22, 24
 Rehberg, P. B., 72, 82, 110, 116
 Reichstein, T., 677, 695
 Reid, D. F., 670, 695
 Reifenstein, E. C., 300, 306, 640, 650
 Reilly, C., 449, 461
 Reimer, A., 212, 216
 Rein, F. H., 142, 150
 Rein, H., 10, 11, 12, 13, 15, 21, 22, 24, 27, 28, 33, 51, 57, 137, 138, 145, 146, 147, 149
 Reinbach, H., 284, 307
 Reinemund, K., 665, 693
 Reinhardt, W. O., 544, 556
 Reis, G. von, 108, 116
 Reiss, F., 298, 308, 319, 320, 335
 Rejtö, K., 469, 481
 Reller, H. C., 587
 Renshaw, B., 52, 57
 Renyi, S. G., 345, 361
 Reppert, E., 444, 462
 Ressmann, A. C., 618, 656
 Rewald, B., 488, 491
 Rex, R. O., 111
 Rey, E., 326, 335
 Rey, J., 45, 57
 Reyersbach, G. C., 633, 656
 Reynolds, S. R. M., 79, 80, 102, 103, 106, 111, 117, 630, 656
 Reynolds, T., 213, 216, 218
 Rhian, M., 644, 655
 Rhoads, C. P., 666, 676, 688, 696
 Ribuffo, A., 479, 480, 481
 Rice, F. A. H., 641, 642, 643, 661
 Richards, A. N., 97, 111
 Richards, R. L., 78, 85, 117, 258, 268
 Richardson, H. B., 235, 236, 240, 242
 Richardson, L. R., 652
 Richert, D. A., 577, 579
 Richter, C. P., 17, 18, 19, 20, 23, 24, 174, 196, 532, 561
 Richter, R. B., 501, 511
 Richter, W., 174, 183, 186, 198, 199
 Rickes, E. L., 677, 695
 Ricketts, C. R., 318, 325
 Riddle, J. M., 646
 Ridley, H. N., 604, 656
 Riemenschneider, R. W., 681
 Riley, J. F., 95, 449
 Riley, V., 539
 Riley, V. T., 540, 561
 Rimington, C., 550, 560, 621, 656
 Ring, J. R., 192, 198
 Ringel, S. J., 356, 363
 Ringier, B. H., 683, 693
 Rinkel, M., 104, 115, 164, 169, 197
 Rittenberg, D., 685, 695
 Ritter, H. B., 436, 462
 Ritter, W. L., 633, 656
 Ritz, N. D., 535, 561
 Rivers, W. H. R., 136, 150
 Rivoire, R., 546, 561
 Robb, G. P., 90, 118
 Robb-Smith, A. H. T., 433, 437, 438, 447, 462
 Robert, P., 42, 43, 57, 228, 231, 545, 561
 Roberts, E., 341, 342, 347, 348, 364, 574, 575, 579, 604, 656
 Roberts, I., 43, 59
 Roberts, J. B., 40, 54
 Roberts, J. S., 517, 556
 Roberts, L. J., 681, 689
 Robertson, A., 527, 553, 630, 659
 Robertson, A. M., 630, 659
 Robertson, J. D., 438, 456
 Robertson, R. F., 681, 695
 Robertson, W. B., 425, 426, 429, 431, 462
 Robertson, W. van B., 394, 416
 Robin, M., 305, 307
 Robinson, A., 655
 Robinson, P., 211, 212, 219
 Robinson, S., 213, 217, 218, 219, 248, 260, 265, 267, 268, 282, 282
 Robinson, W. D., 430, 457
 Roboz, E., 670, 693
 Robson, H. N., 453, 462
 Rocha e Silva, M., 96, 111, 117
 Roddy, W. T., 347, 364, 409, 416
 Rodzevitch, M., 520, 553, 555
 Roe, E. M. F., 605, 651
 Röhm, F., 321, 324, 326, 335
 Rogers, H. J., 432, 461, 462
 Rogers, M. P., 71, 117
 Rollhäuser, H., 5, 6, 7, 8, 449, 462
 Roma, M., 362, 494, 500, 501, 502, 503, 504, 511
 Romano, J., 87, 88, 89, 118
 Ronchese, F., 621, 632, 656
 Rony, H. R., 298, 302, 308, 626, 657
 Roome, N. W., 101, 117
 Root, H. F., 233, 238, 241
 Ropes, M. W., 425, 426, 429, 431, 462
 Ropshaw, H. S. J., 520, 522, 561
 Roques, E., 45, 57
 Rose, B., 99, 117
 Rose, C. S., 673, 690
 Rose, W. C., 368, 390, 664, 695
 Roseman, S., 424, 427, 428, 432, 459, 462
 Rosenberg, E., 641, 661
 Rosen, F., 669, 695
 Rosenberg, I., 220
 Rosenberg, I. N., 544, 561
 Rosenblueth, A., 86, 110, 167, 193
 Rosenblum, L. A., 670, 692
 Rosendal, T., 12, 25
 Rosenfeld, H., 318, 335
 Rosenthal, S. R., 96, 115, 142, 150
 Rosenthal, T. B., 503, 504, 512
 Ross, C. A., 101, 115
 Rossiter, R. S., 705, 706
 Rossmeisl, E., 425, 426, 462
 Rost, E., 657
 Rost, G. A., 22, 25
 Roth, E., 625, 657
 Roth, G. M., 80, 94, 95, 108, 112, 114, 117, 254, 256, 268
 Roth, I., 545, 561
 Roth, J. S., 632, 634, 655
 Rothman, S., 12, 25, 27, 28, 29, 32, 33, 35, 36, 38, 39, 40, 41, 42, 44, 45, 46, 47, 48, 49, 50, 52, 54, 57, 58, 59, 80, 88, 90, 97, 98, 107, 108, 111, 117, 123, 125, 127, 129, 130, 131, 132, 135, 136, 137, 138, 140, 141, 142, 143, 144, 145, 151, 160, 161, 163, 164, 166, 167, 168, 169, 170, 171, 172, 173, 176, 177, 187, 188, 189, 190, 192, 194, 195, 198, 202, 203, 204, 205, 207, 208, 209, 210, 211, 213,

- 219, 221, 222, 228, 231, 233, 234, 236, 238, 239, 242, 243, 258, 261, 267, 269, 296, 297, 298, 299, 300, 302, 304, 305, 306, 308, 312, 313, 314, 316, 317, 318, 321, 322, 323, 325, 328, 329, 330, 334, 335, 336, 351, 359, 364, 367, 368, 372, 374, 375, 377, 378, 381, 387, 388, 389, 465, 466, 467, 468, 469, 471, 481, 486, 487, 489, 491, 494, 495, 498, 500, 503, 504, 508, 510, 513, 519, 522, 528, 533, 534, 535, 546, 547, 548, 550, 552, 554, 555, 561, 563, 571, 574, 578, 579, 580, 581, 582, 583, 589, 600, 623, 640, 642, 643, 649, 657, 660, 668, 695, 704, 706
- Rothstein, M., 499, 513
 Roucayrol, J. C., 37, 38, 58
 Rourke, G. M., 499, 513
 Rous, P., 71, 73, 105, 117
 Routh, J. I., 621, 648, 673, 695
 Rouzand, J. J., 470, 479
 Rowntree, L. G., 516, 561
 Royo, F., 643, 656
 Rubin, J., 272, 282, 552, 561
 Rubin, L., 98, 117, 378, 389, 522, 550, 552, 555, 561, 586, 598, 600
 Rudall, K. M., 344, 345, 346, 361, 364, 370, 605, 612, 615, 649, 651
 Rudloff, E. von, 315, 321, 333, 335
 Ruedemann, R., 49, 54
 Ruiter, M., 706
 Runne, E., 542, 556, 633, 651, 688, 691
 Rupp, J. J., 670, 689
 Rusch, H. P., 670, 694
 Russel, W. L., 523, 561
 Ruzsnyak, S., 679, 695
 Ruzicka, L., 326, 327, 335, 336
 Rydon, H. N., 573, 578, 704, 705, 705
 Rygh, O., 681, 697
 Ryneerson, E. H., 253, 269
 Rýssel, T. G. van., 706
- Saalfeld, E., 622, 657
 Sabouraud, R., 630, 639, 657
 Sacerdote de Lustig, E., 447, 459, 572, 578
 Sach, P. M., 534, 553
 Sachs, B., 630, 657
 Sack, S. S., 44, 59
 Sack, W. T., 139, 140, 151
 Sacks, B., 102, 117
 Säberg, I., 649
 Saenz-Herrera, C., 542, 560, 624, 647
 Saiki, A. K., 205, 219
 Sainton, P., 627, 657
 Saint-Phalle, M. de, 318, 335
 Saito, K., 161, 198
 Sakamoto, H., 158, 161, 195
 Sakata, S., 498, 513
 Salkowsky, E., 486, 491
 Saller, K., 518, 561
 Sallman, B., 432, 462
 Salmon, A. A., 640, 647
 Salmon, K., 673, 694
 Salmon, R. J., 444, 463
 Salmon, W. D., 670, 695
 Salo, T. P., 396, 413
 Samberger, F., 186, 198
 Samitz, M. H., 670, 698
 Sammartino, U., 372, 389
 Sampson, W. L., 543, 563
 Sams, W. M., 380, 389
 Sanchez, S., 289, 291, 293, 294, 307, 310, 333
 Sandberg, M., 103, 116
 Sanders, A. G., 76, 117
 Sanders, F. K., 121, 126, 127, 130, 131, 134, 137, 147, 151
 Sandison, J. C., 64, 117
 Santori, G., 486, 491
 Sargent, F., 211, 212, 219
 Sarma, P. S., 669, 693
 Sarnoff, J., 89, 117
 Sarre, H., 32, 45, 56
 Saudek, J., 611, 657
 Savit, J., 52, 59
 Sawhill, J., 670, 692
 Sawyer, W. H., 35, 58
 Scarborough, H., 679, 695
 Schaad, J. A., 397, 404, 405, 413
 Schaaff, F., 45, 58, 187, 188, 192, 198, 202, 203, 205, 208, 209, 210, 213, 215, 216, 219, 221, 228, 231, 233, 234, 238, 243, 258, 269, 289, 290, 295, 302, 308, 314, 316, 321, 323, 324, 325, 330, 335, 367, 368, 374, 389, 465, 466, 467, 468, 469, 471, 477, 481, 486, 487, 491, 494, 495, 496, 498, 500, 503, 504, 508, 513, 520, 521, 532, 561, 580, 581, 582, 583, 642, 657, 704, 706
 Schade, H., 3, 8, 11, 25, 221, 231, 534, 556
 Schafer, E. P. S., 103, 117
 Schaffer, J., 284, 285, 288, 308, 310, 335
 Schamberg, I. L., 632, 657
 Schaumann, K., 532, 561
 Schein, M., 630, 657
 Schenk, P., 210, 219
 Scheunert, A., 204, 219
 Schiefferdecker, P., 180, 182, 198, 603, 657
 Schill, E., 115, 175, 198
 Schiller, C., 398, 413
 Schiller, S., 441, 452, 454, 462
 Schindel, L., 320, 323, 333, 335
 Schkloven, N., 102, 109
 Schlegel, J. U., 209, 219
 Schleich, H., 396, 414
 Schlenck, F., 668, 690
 Schlomka, G., 485, 490, 495, 510
 Schlosberg, H., 18, 25
 Schloss, S., 102, 117
 Schlossmann, H., 50, 55
 Schmid, G., 39, 58
 Schmid, M., 229, 230, 231
 Schmid, R., 471, 480
 Schmidt, C. L. A., 337, 339, 342, 364
 Schmidt, H. E., 619, 657
 Schmidt, M. B., 302, 308
 Schmidt, P., 116
 Schmidt, P. W., 223, 224, 227, 228, 231
 Schmidt, V., 624, 657
 Schmidt, W. J., 344, 364
 Schmidt-Nielsen, K., 310, 311, 335
 Schmitt, F. O., 397, 401, 407, 408, 409, 414, 416, 422, 447, 457, 458
 Schneck, J. H., 134, 151
 Schneider, D., 103, 117
 Schneider, H., 523, 556, 670, 695
 Schöberl, A., 356, 357, 364, 643, 657
 Schönberg, A., 289, 290, 291, 293, 294, 298, 304, 307
 Schoenfeld, R., 321, 334
 Schönheimer, R., 323, 335, 487, 491
 Schoenheimer, R., 685, 695
 Schöpp, K., 679, 693
 Scholtz, W., 221, 226, 232
 Schoor, H. C., 500, 501, 503, 504, 505, 510
 Schorr, E., 61, 117
 Schour, I., 593, 595, 597, 599
 Schrafl, A., 546, 554
 Schreiber, E., 42, 58
 Schreiber, N. E., 42, 43, 54
 Schreiner, A. W., 670, 695
 Schroeder, H., 534, 561
 Schubert, M., 397, 414, 425, 434, 456, 459
 Schultz, A. H., 604, 657
 Schultz, F. N., 223, 232
 Schulze, E., 326, 335
 Schulze, H., 326, 335
 Schulze, R., 552, 557
 Schulze, W., 210, 219, 221, 232, 262, 268, 580, 581, 582, 583
 Schumacher, G. A., 134, 135, 151
 Schumacher, J., 32, 59, 344, 351, 365, 367, 373, 375, 390, 486, 491
 Schumann, R., 205, 219
 Schuppli, R., 227, 232, 467, 481
 Schur, H., 288, 308
 Schurmeyer, A., 238, 242
 Schuster, P., 665, 694
 Schwanitz, J., 606, 607, 611, 657
 Schwartz, H. G., 17, 20, 25, 173, 174, 176, 198
 Schwartz, I. L., 204, 219
 Schwartz, J. H., 625, 657
 Schwartz, L., 534, 535, 560, 562, 633, 657
 Schwartz, R., 501, 512
 Schwarz, G., 639, 657
 Schwarz, L., 642, 643, 657
 Schweder, M., 233, 236, 238, 241
 Schweigert, B. S., 662, 673, 690
 Schwemmler, B., 681, 695

- Schwenkenbecher, A., 27, 35, 45,
58, 201, 202, 213, 219, 238, 243
- Schwieger, A., 488, 491
- Scott, C. M., 116
- Scott, J. C., 163, 198, 265, 269
- Scott, W. O., 40, 53
- Scow, R. O., 398, 416
- Scroggie, A. E., 96, 117
- Sealock, R. R., 665, 695
- Seaman, A., 677, 697
- Seastone, C. V., 431, 458
- Sebrell, W. H., 666, 670, 676, 684,
690, 693, 695
- Sebruyns, M., 644, 657
- Seeberg, G., 441, 462
- Seegers, W. H., 684, 696
- Segall, A., 606, 657
- Seifter, J., 430, 442, 449, 451, 455,
456, 463
- Seitz, L., 182, 198
- Seitz, P. F. D., 139, 151
- Sekora, A., 400, 415
- Selle, W. A., 37, 57, 58
- Sellei, C., 470, 471, 481
- Sellheim, H., 630, 657
- Selye, H., 300, 308, 441, 454
- Semina, L. A., 348, 363, 574, 578
- Senear, F. E., 71, 118
- Senior, F. A., 87, 104, 108
- Serra, V., 69, 118
- Serrati, B., 288, 291, 295, 302,
303, 304, 305, 308
- Seville, R. H., 451, 463
- Sevringhaus, E. L., 669, 691
- Sexsmith, E., 704, 706
- Seymour, R. J., 625, 630, 657
- Shakir, M. H., 541, 554
- Shank, R., 42, 43, 55
- Shapiro, A. L., 144, 145, 151, 314,
318, 319, 320, 335
- Shapiro, E., 625, 630, 658
- Shapiro, R., 444, 463
- Sharlit, H., 224, 227, 228, 232,
522, 562
- Sharp, M. E., 305, 622, 649
- Shatamova, L. V., 666, 694
- Shaw, H. C., 318
- Shaw, L. A., 45, 58, 580, 581, 582,
583
- Shea, E., 238
- Sheard, C., 108, 113, 117, 244,
247, 248, 250, 253, 254, 255,
256, 258, 259, 267, 268, 269,
516, 518, 554, 562
- Sheehan, H. L., 544, 562
- Sheer, M., 224, 227, 228, 232
- Sheline, G. E., 688, 694, 696
- Shelley, W. B., 34, 42, 47, 49, 51,
52, 53, 58, 140, 143, 150, 151,
160, 168, 172, 181, 183, 184,
186, 187, 188, 190, 191, 192,
194, 198, 199, 222, 232, 272,
275, 277, 278, 279, 280, 281,
282, 283, 378, 389, 468, 481
- Shepardson, H., 625, 630, 658
- Sherman, H., 35, 59
- Sherman, W. B., 67, 110
- Sherren, J., 136, 150
- Sherrington, C. S., 139, 151
- Sherskul'skaya, L. V., 575, 577
- Shilkret, H. H., 51, 58
- Shizume, K., 545
- Shore, A., 392, 393, 405, 407,
409, 410, 415, 701, 706
- Short, J. J., 104, 114
- Shulman, I., 104, 116
- Shute, E. V., 683, 696
- Shute, W. E., 683, 696
- Sicher, G., 467, 469, 470, 477, 481
- Sieburg, E., 209, 219
- Siemens, H. W., 42, 58
- Sigel, H., 135, 137, 151
- Sikes, D., 680
- Silber, R. H., 673, 696
- Silberstein, H. E., 665, 695
- Silbert, S., 681, 695
- Silver, H., 356, 362
- Silver, S. D., 30, 41, 54
- Silver, V. E., 508, 514
- Silverman, S. H., 641, 660
- Silvers, S., 209, 219, 478, 481
- Silvers, S. H., 221, 222, 226, 231
- Simeone, F. A., 89, 117
- Simmonet, H., 627, 657
- Simms, H. D., 543, 560
- Simois, R. L., 490
- Simons, R. D. G. P., 277, 283
- Simpson, W. L., 429, 463
- Simuango, S., 4, 8
- Sinclair, D. C., 125, 133, 142,
151, 152
- Sinclair, H. M., 681
- Sinclair, R. G., 488, 491
- Singal, S. A., 674, 696
- Singer, L., 541, 542, 554, 562,
672, 696
- Singher, H. O., 666, 696
- Sirdar, J. N., 624, 644, 645
- Sister Alfred de Marie, 545, 559
- Siu, G. H., 359, 364
- Sizer, I. W., 395, 397, 415, 416,
530, 562
- Sjögren, H., 681, 696
- Sjöstrand, F., 108, 116
- Sjöstrand, T., 107, 118
- Sjollema, B., 541, 562
- Skelton, F. R., 683, 696
- Skelton, H. P., 494, 497, 498, 500,
513
- Skidmore, B. E., 648
- Skouby, A. P., 122, 123, 142, 143,
148, 151
- Slack, H. B., 394, 416
- Slade, J. G., 92, 111
- Slanetz, C. A., 681
- Slaunwhite, W. R., 527, 560
- Slepyan, A. H., 644, 658
- Slinger, W., 670, 695
- Slinger, W. N., 644, 658
- Smedley-MacLean, I., 318, 335,
484, 491
- Smiljanic, A. M., 205, 207, 209,
219, 314, 317, 319, 320, 335,
336, 532, 561
- Smith, B. F., 665, 697
- Smith, C., 372, 375, 376, 389
- Smith, C. C., 41, 59
- Smith, D. E., 157, 189, 194
- Smith, E. L., 677, 689, 696
- Smith, F., 73, 105
- Smith, H. P., 684, 696
- Smith, H. W., 52, 53
- Smith, J. H., 111
- Smith, L. H., 140, 151
- Smith, M. I., 644, 660
- Smith, P. H., 625, 626, 628, 629,
645
- Smith, P. N., 105, 114
- Smith, R. P., 642, 643, 661
- Smith, S., 641, 642, 658
- Smith, S. E., 658
- Smith, S. G., 670, 696
- Smith, S. W., 541, 562
- Smith, V. W., 535, 554, 611, 647
- Smithwick, R. H., 86, 112
- Smuts, D. B., 623, 658, 664, 696
- Smyth, E. M., 423, 429, 460
- Smyth, J. R., 518
- Snapp, R. H., 238, 243
- Snapper, L., 210, 219
- Snell, E. E., 673, 676, 690, 694,
695
- Snellman, O., 424, 454
- Snider, B. L., 489, 491
- Snider, R., 488, 490
- Snow, J. S., 302, 308, 614, 658
- Snowman, R. T., 186, 199, 216,
219
- Snyder, F. H., 325
- Snyderman, S. E., 670
- Sobel, E. H., 4, 8
- Sobel, H., 327, 328, 329, 336
- Sobotka, K., 535, 560
- Sodeman, W. A., 239, 243, 546,
562
- Soderstrom, G. F., 238, 243, 252,
257
- Soffer, L. J., 440, 459, 681, 695
- Sokolowsky, A., 603, 604, 658
- Sollmann, T., 42, 54, 104, 118
- Solomon, H., 683, 693
- Sonne, C., 264, 269
- Sonnenschein, R. R., 86, 114,
140, 142, 150, 151, 166, 167,
168, 171, 194, 195, 199
- Sookne, A. M., 407, 413
- Souter, A. W., 684, 693
- Spain, D. M., 444, 445, 463
- Spalteholz, W., 61, 118
- Speakman, J. B., 354, 355, 356,
357, 364
- Spealman, C. R., 257, 269
- Spector, H., 211, 216, 219
- Spector, W. G., 434, 463
- Spencer, B., 645, 658
- Spencer, G. A., 534, 541, 554, 562
- Spencer, H. C., 45, 53
- Spencer, M. C., 289, 291, 293,
294, 307, 310, 333
- Sperling, F., 192, 199
- Sperling, G., 156
- Sperry, R. W., 120, 151
- Spiera, M., 470, 471, 481
- Spies, T. D., 104, 118, 670, 696
- Spillmann, M., 326, 335
- Spira, L., 645, 658
- Spiro, D., 630, 657

- Spitta, O., 213, 219
 Spitzer, R. R., 658
 Spoor, H. J., 546, 562
 Sprunt, D. H., 441, 463
 Squire, J. R., 318, 325, 335
 Srere, P. A., 485, 487, 491, 577, 579
 Ssoblewa, N. G., 613, 658
 Stacey, M., 424, 463
 Staff, H., 216, 220
 Stahel, W., 681, 696
 Stahl, W. H., 359, 364
 Stanger, D. W., 328, 335
 Stanier, J. E., 426, 453, 461
 Stanley, M., 430, 457
 Stanley, W. C., 18, 25
 Stanley, W. M., 326, 336
 Starkenstein, E., 37, 48, 58
 Starling, E. H., 72, 118, 604, 658
 Stary, Z., 540, 562
 Stead, E. A., Jr., 75, 87, 88, 89, 101, 102, 114, 118
 Stearner, S. P., 538, 562
 Stearns, M. L., 442, 463
 Steckel, 139, 151
 Steenbock, H., 651, 656, 670, 679, 681, 685, 688, 692, 695, 696
 Stein, H. J., 670, 698
 Stein, I. D., 32, 58, 87, 118
 Stein, I. F., 626, 658
 Stein, R. O., 627, 631, 658
 Stein, W. H., 395, 411, 412, 413, 416
 Steinberg, D. L., 666, 692
 Steiner, P. E., 328, 336
 Steinhardt, J., 228, 232
 Steinhardt, R. G., Jr., 404, 416
 Steinke, O., 45, 55
 Stellar, E., 145, 146, 150
 Stephenson, M. L., 573, 579, 704, 707
 Stepto, R. C., 444, 462
 Stern, F., 494, 498, 501, 503, 508, 509, 513
 Sternberg, T. H., 466, 467, 471, 478, 481
 Stetson, R. H., 147, 151
 Steuber, M., 204, 219
 Stevens, J. R., 670, 693, 696
 Stewart, C. T., 444, 463
 Stewart, G. N., 104, 118
 Stewart, J. D., 499, 513
 Stewart, W. B., 186, 199, 216, 219
 Stiefeler, G., 304, 308
 Stiffel, C., 41, 55
 Stiller, E. T., 670, 696
 Stinchfield, F., 216, 220
 Stirn, F. E., 541, 562
 Stocken, L. A., 704, 706
 Stoddard, F. J., 625, 658
 Stöhr, P., Jr., 180, 189, 199
 Stokes, H. J., 139, 151
 Stokes, J., 140, 151
 Stokhuyzen, A. W., 627, 658
 Stokinger, H. E., 664
 Stokstad, E. L. R., 667, 677, 689, 692, 695
 Stolfi, G., 526, 559
 Stone, C. S., 67, 104, 112
 Stone, L. S., 549, 562
 Stone, R. E., 104, 118
 Storey, W. F., 593, 595, 596, 600
 Stoughton, R. B., 397, 416, 433, 436, 437, 452, 463, 573, 579, 699, 701, 705, 706
 Stoves, J. L., 351, 356, 359, 364, 365
 Straith, R. E., 398, 413
 Strakosch, E. A., 32, 47, 48, 49, 51, 58
 Strang, J. M., 108, 110
 Strangeways, D. H., 622, 658
 Strangeways, T. S. P., 385, 389
 Strasser, A., 107, 118
 Strassmann, E. O., 626, 658
 Straub, W., 644, 658
 Stricker, S., 89, 118
 Ströbele, R., 665, 693
 Strohl, A., 37, 38, 58
 Strong, F. M., 669, 690
 Struck, H. C., 681, 690
 Strzyzowski, C., 645, 658
 Stuart, H. C., 4, 8
 Stubbings, R. L., 396, 416
 Stubbs, A. L., 541, 554
 Studer, A., 382, 383, 389
 Sturges, S., 485, 486, 491
 Subbarow, Y., 543, 556
 Sugiura, H. T., 632, 634, 655
 Sugiura, K., 532, 562
 Suksta, A., 670, 698
 Sulkowitch, H. W., 300, 306
 Sullivan, M., 543, 562, 658, 666, 672, 673, 674, 688, 696
 Sullivan, M. B., 203, 204, 207, 208, 219, 335, 372, 389, 574, 579
 Sullivan, R. D., 634, 659
 Sulman, F. G., 544, 562
 Sulzberger, M. B., 29, 30, 32, 33, 34, 41, 44, 47, 55, 56, 58, 59, 97, 99, 109, 118, 141, 152, 154, 159, 160, 177, 190, 194, 199, 225, 230, 258, 268, 270, 272, 277, 280, 281, 282, 283, 293, 295, 306, 310, 318, 633, 659
 Sumegi, I., 545, 561
 Summers, V. K., 625, 626, 628, 629, 659
 Summerson, W. H., 217, 523, 528, 529, 530, 558
 Sumner, F. B., 520, 562
 Sundberg, C., 465, 481
 Sunderman, F. W., 235, 236, 243
 Suntzeff, V., 348, 364, 381, 389, 484, 485, 491, 492, 494, 501, 502, 503, 505, 510, 511, 513, 514, 569, 577, 699, 701, 705, 707
 Suskind, R. R., 310, 311, 330, 331, 335, 336
 Sutton, R. L., Jr., 586, 594, 596, 600
 Suzuki, S., 296, 302, 308
 Sydenstricker, V. P., 674, 696
 Sylvén, B., 434, 443, 449, 463, 613, 659
 Szadurski, J. A. R., 261, 267
 Szakall, A., 229, 232
 Szasz, T. S., 630, 659
 Szczesniak, A. S., 35, 59
 Szent-Györgyi, A., 534, 546, 562, 677, 679, 695, 697
 Szép, Ö., 641, 643, 659
 Szirmai, J. A., 394, 398, 416
 Szodoray, L., 154, 187, 199, 367, 390, 509, 514, 699, 704, 707
 Szörényi, E., 575, 579
 Tabor, H., 670, 693
 Taboury, M. M. F., 644, 659
 Tachau, H., 207, 217
 Tada, K., 644, 659
 Tadokoro, T., 621, 659
 Täufel, K., 328, 335
 Takagaki, T., 168, 171, 172, 199
 Takahara, K., 157, 158, 199
 Takats, G. de, 104, 109
 Takino, M., 303, 308
 Talbert, G. A., 202, 205, 208, 209, 215, 216, 217, 219, 220, 222, 232, 478, 481
 Talbot, N. B., 213, 214, 218, 265, 268
 Talbot, S. A., 101, 113
 Talbott, J. H., 265, 269, 509, 512, 514, 681, 693
 Tamura, S., 621, 659
 Tankel, H. I., 180, 199
 Tarantino, C., 348, 365
 Tarchanoff, J., 18, 25
 Tarras-Wahlberg, B., 96, 118
 Tarrell, E. S., 213, 219
 Taschdjian, C. L., 359, 364
 Tattersall, R. N., 450, 463
 Tatum, E. L., 670, 695
 Taub, S. J., 685, 697
 Taubenhause, M., 444, 445, 463
 Tauber, E. B., 681, 697
 Taussig, J., 516, 562
 Taylor, A. C., 537, 562
 Taylor, B., 444, 445, 463
 Taylor, E., 310, 311, 335
 Taylor, H., 681, 690
 Taylor, H. C., 666, 696
 Taylor, H. L., 69, 113, 265, 269
 Taylor, J. D., 488, 491
 Taylor, N. B., 86, 189, 193, 264, 266, 663, 689
 Taylor, R. M., 75, 85, 110
 Tchen, P. K., 327, 334
 Teilum, G., 451, 463
 Templeton, H., 632, 659
 Teply, L. J., 669, 693
 Tershakovec, G. A., 445, 460
 Thaller, G., 535, 558
 Thannhauser, 559
 Thauer, R., 199, 244, 269
 Thaysen, J. H., 204, 219
 Theis, E. R., 392, 393, 394, 396, 404, 405, 409, 415, 416, 494, 495, 503, 513
 Thélin, F., 675, 697
 Thibierge, G., 639, 659

- Thigpen, L. W., 604, 659
 Thing, E., 544
 Thöle, 135, 152
 Thomas, E. W. P., 683, 690
 Thomas, K., 202, 220
 Thomas, L. C., 604, 656
 Thomas, V., 37, 56
 Thompson, H. C., Jr., 587, 589
 Thompson, R. G., 41, 55
 Thompson, R. H. S., 89, 568, 569, 570, 571, 576, 578, 579, 703, 704, 706, 707
 Thompson, W. S., 105, 115
 Thomson, J. F., 52
 Thomson, M. L., 277, 283
 Thoreaux, 397, 416
 Thorn, G. W., 517, 544, 557, 562
 Thorner, J. E., 640, 659
 Threefoot, S. A., 510
 Thuringer, J. M., 586, 587, 589, 591, 597, 600
 Thurmon, F. M., 161, 199, 211, 220, 222, 232, 489, 491
 Thyresson, N., 612, 613, 614, 633, 646, 659
 Tickner, A., 89
 Tiedt, J., 321, 336
 Tietz, E. B., 614, 659
 Timmis, G. M., 605, 651
 Timpano, P., 638, 659
 Tisdall, F. F., 666, 697
 Tishkott, G. H., 347, 364
 Todd, T. W., 516, 562
 Todd, W. R., 688, 697
 Toennies, G., 390
 Törö, E., 443, 458
 Török, L., 99, 118
 Tolmach, J. A., 541, 554
 Tommasi, L., 632, 659
 Toolan, H. W., 591, 600
 Topley, E., 318, 325, 335
 Toropoff, T., 10, 22
 Tushima, E., 470, 481
 Totter, J. R., 676, 690
 Tower, S. S., 90, 118, 121, 125, 126, 131, 152
 Townsend, F., 354, 356, 357, 364
 Traube-Mengarini, M., 37, 59
 Trautmann, A., 659
 Travis, R. C., 9, 24
 Treitman, S. S., 485, 487, 491, 577, 579
 Trice, E. R., 41, 55
 Trimble, H., 469, 471, 481
 Trimble, H. C., 467, 469, 470, 480
 Trinkaus, J. P., 545, 562
 Trolle, C., 235, 243
 Trotter, M., 615, 616, 617, 618, 619, 621, 649, 659
 Trowell, H. C., 542, 562
 Trueblood, D. V., 535, 563
 Truesdail, J. H., 672, 697
 Truffi, G., 303, 304, 308, 633, 660
 Truter, E. V., 314, 315, 316, 321, 329, 335
 Tsao, S. N., 680, 691
 Tschernjachiwsky, A., 621, 660
 Tschernogubow, N. A., 638, 660
 Tschesche, R., 326, 336
 Tsujimoto, M., 328, 336
 Tufts, E. V., 688, 697
 Tui, C., 4, 8
 Tulane, V. J., 352, 365
 Tunbridge, R. E., 422, 438, 448, 450, 457, 463
 Turner, F. C., 539, 540, 556
 Turrell, E. S., 265, 268
 Tustanovskii, A. A., 409, 416, 447, 461
 Tuttle, L. C., 672, 693
 Tytell, A. A., 416
 Tyvand, R., 220
 Uehlinger, E., 46, 55, 547, 557
 Ugami, H., 621, 659
 Ugi, I., 222, 230
 Ugo, A., 492
 Umberger, E. J., 31, 43, 47, 48, 49, 51, 52, 56
 Umrath, K., 90, 94, 113, 118, 130, 143, 148, 152
 Underhill, F. P., 668, 690
 Underwood, G. B., 379, 380, 388
 Unger, I., 544, 557
 Unna, K., 543, 563, 645, 666, 670, 689, 696
 Unna, P., 310, 324, 336, 450, 463
 Unna, P., Jr., 48, 59
 Unna, P. G., 32, 59, 192, 199, 235, 239, 243, 247, 269, 344, 351, 360, 365, 367, 372, 373, 375, 390, 486, 491, 699, 707
 Uprus, V., 179, 199
 Upshaw, B. Y., 235, 243
 Upton, A. C., 444, 463
 Urbach, E., 341, 342, 347, 365, 467, 469, 470, 471, 477, 481, 483, 491, 493, 495, 496, 499, 500, 508, 509, 514, 680, 681, 697
 Urbach, F., 33, 54
 Usher, B., 210, 220
 Ussing, H. H., 35, 55, 59
 Uyeno, K., 166, 196
 Valette, F., 27, 38, 46, 53
 Valette, G., 41, 46, 49, 59
 Vallance-Owen, J., 191, 199
 Vámos, L. von, 704, 707
 Vanamee, P., 394, 397, 416, 447, 463
 Van Arman, C. G., 528, 563
 Van Cleve, J., 42, 43, 54
 Van der Burg, A. P., 46, 59
 Van Duijn, P., 521
 Van Dyke, D. C., 639, 660
 Van Lier, E. H. B., 423, 463
 Van Scott, E. J., 204, 220, 348, 365, 370, 486, 492, 502, 509, 514, 533, 563, 574, 579, 699, 704, 707
 Van Wagenen, G., 441, 447, 456
 Vasti, A., 233, 234, 235, 243
 Vaubel, E., 448, 463
 Vaughan-Morgan, J., 641, 646
 Veer, W. L. C., 677, 697
 Veiel, E., 107, 116
 Velick, S. F., 316, 336
 Velluz, L., 314, 321, 323, 329, 332, 336
 Veraguth, O., 18, 25
 Vermeer, D. J. H., 208, 220, 227, 228, 229, 232
 Verne, J., 37, 38, 58
 Vértes, T., 509, 514, 704, 707
 Verzár, F., 660
 Vickery, H. B., 353, 361
 Vietmeyer, O., 326, 334
 Viets, H. R., 174, 195
 Vignes, R., 646
 Vignolo-Lutati, K., 634, 660
 Vilter, C. F., 677, 697
 Vilter, R. W., 670, 677, 695, 697
 Vimtrup, B., 76, 118
 Violante, A., 104, 114
 Vitte, G., 643, 660
 Vlès, F., 492
 Vogelsang, A. B., 683, 696
 Voit, E., 202, 203, 220, 374, 390, 617, 660
 Volk, M. C., 582, 583, 583
 Volkmann, R. von, 594, 596, 597, 600
 Vorhaus, M. G., 623, 660, 676, 697
 Vos, E. A., 31, 48, 49, 51, 52, 56
 Voser, W., 327, 336
 Voss, H. E., 41, 59, 302, 308
 Wachtel, L. W., 688, 697
 Wachter, R., 45, 55
 Wacker, L., 302, 307
 Wada, M., 161, 162, 166, 167, 168, 171, 172, 183, 184, 193, 199
 Waddell, J., 688, 692
 Wadel, J., 630, 660
 Waelsch, L., 182, 199
 Wagenaar, J. H., 170, 199
 Wagner, H. N., Jr., 18, 25
 Wagner-Jauregg, T., 665, 693
 Wahlgren, V., 514
 Waisman, H. A., 654, 665, 666, 673, 674, 690, 694, 697
 Waitkoff, H. K. W., 411, 414
 Wakeman, A. M., 203, 204, 218
 Waksman, B. H., 394, 417
 Wald, G., 634, 660, 680, 697
 Walker, A., 489, 491
 Walker, A. D., 677, 689
 Walker, A. E., 121, 152
 Walker, S. A., 41, 59, 640, 660
 Wallenstern, L. von, 611, 660
 Walshe, F. M. R., 90, 118, 131, 137, 152
 Walter, C. W., 59, 87, 88, 89, 118
 Walzer, A., 44, 59
 Wang, G. H., 174, 199
 Wanger, J. O., 535, 553
 Warburg, O., 477, 481, 482, 665, 668, 697
 Waring, H., 544, 563
 Warkany, J., 382, 390
 Warner, C., 66, 114
 Warner, E. D., 684, 696
 Warrack, G. H., 397, 416, 431, 433, 461
 Warren, J. V., 87, 88, 89, 118

- Warren, L. H., 534, 535, 560, 562
 Warren, S., 537, 558, 639, 653
 Washburn, S. L., 325, 336
 Waterlow, J. C., 270, 281, 282
 Waterman, N., 503, 514
 Wather, R., 45, 54
 Watrous, R. M., 643, 660
 Watson, E. M., 423, 440, 453, 461, 464
 Watson, S. J., 534, 555
 Waugh, D. F., 447, 464
 Waugh, W. A., 677, 693
 Way, S. C., 154, 192, 199, 535, 563
 Weatherford, C., 35, 55
 Weber, M., 644, 653
 Webster, M. E., 430, 456
 Wechselmann, W., 235, 242
 Weddell, G., 122, 123, 124, 125, 126, 133, 142, 145, 150, 152, 189, 200
 Wehrli, H., 679, 693
 Weider, F. V., 681, 697
 Weidinger, A., 355, 362, 365, 367, 388
 Weidman, F. D., 272, 277, 281, 283, 699, 706
 Weil, K., 621, 660
 Weiner, J. S., 158, 159, 160, 200, 202, 210, 211, 220
 Weinmann, J. P., 593, 595, 597, 599
 Weinstein, I., 32, 58
 Weinstock, M., 329, 333, 681, 692
 Weir, C. E., 404, 405, 417
 Weiss, H. V., 508, 514
 Weiss, J., 510, 513
 Weiss, L., 621, 654
 Weiss, S., 45, 58, 72, 75, 90, 101, 102, 104, 112, 114, 118, 119, 580, 581, 582, 583
 Weitkamp, A. W., 314, 315, 316, 317, 319, 320, 335, 336
 Weitz, J., 136, 147, 152
 Weitzel, G., 316, 321, 324, 325, 336
 Welch, A. D., 543, 563
 Weld, J. T., 611, 652
 Wells, G. C., 421, 436, 437, 451, 452, 459, 463, 464, 704, 705, 707
 Wells, J. A., 47, 57, 96, 105, 109, 111
 Wells, L. J., 549, 563
 Welsh, J. H., 549, 560
 Wenner, H. A., 430, 432, 464
 Wenzel, H. G., 4, 7, 8
 Werder, F. von, 326, 336
 Werne, J., 569, 578
 Werner, B., 458
 Werner, G., 485, 491
 Werner, S. C., 439, 464
 Wertheimer, E., 35, 59
 Werthessen, N. T., 302, 307
 Wertzler, D., 493, 494, 497, 498, 501, 503, 511
 West, G. B., 86, 95, 118, 449
 Westerfeld, W. W., 548, 563, 577, 579
 Westfall, B. B., 644, 660
 Westphal, K., 681, 697
 Weth, G., 94, 114
 Wettstein, A., 679, 693
 Wetzal, N. C., 61, 118
 Weygand, F., 665, 693
 Wheatley, V. R., 312, 313, 314, 323, 324, 325, 327, 328, 329, 330, 334, 336
 Wheelan, F. G., 18, 25
 Wheeler, A. H., 531, 563
 Wheeler, C. E., 547, 563
 Wheeler, G. A., 668, 691, 697
 Wheland, G. W., 357, 365
 Whevell, C. S., 356, 364
 Whipple, G. H., 216, 217, 219
 Whitaker, W. L., 300, 305, 454, 546, 563, 624, 626, 629, 660
 Whitche, C. E., 88, 118
 White, A., 368, 390
 White, A. G., 532, 536, 563
 White, E. A., 685, 697
 White, J. C., 86, 112
 Whitehead, R. W., 614, 658
 Whitehouse, A. G. R., 35, 36, 59, 201, 202, 212, 213, 215, 217, 220, 234, 235, 238, 239, 242, 243
 Whiteley, H. J., 612, 613, 660
 Whiteside, J., 398, 413
 Whitfield, A., 80, 119
 Whitlock, F. A., 534, 563
 Whitman, E. N., 200
 Whittaker, V. P., 576, 579
 Whitteridge, D., 87, 112
 Whittle, C. H., 620, 654
 Whorton, C. M., 445, 460
 Whyte, H. M., 135, 152
 Wibaut, J. P., 634, 653
 Wichman, W., 45, 55
 Wicks, L. F., 484, 485, 492
 Widner, W. R., 595, 596, 599
 Wieland, H., 326, 327, 336
 Wiener, J. S., 268
 Wiese, H. F., 685, 690, 692
 Wigmena, H. G., 677, 697
 Wilcox, J. C., 550, 563
 Wild, R. B., 43, 48, 59
 Wilde, D. S., 64, 111
 Wilder, R. M., 665, 677, 689, 697
 Wile, U. J., 302, 308, 324, 332, 486, 490
 Wiley, F. H., 233, 238, 243
 Wilkerson, V. A., 352, 353, 365
 Wilkins, L., 641, 660, 661
 Wilkins, R. W., 74, 100, 104, 119, 168, 172, 200
 Wilkinson, J. F., 534, 546, 563
 Wilkinson, S., 316, 332
 Williams, A. W., 638, 661
 Williams, B. W., 461
 Williams, C. D., 542, 563
 Williams, D. H., 258, 269
 Williams, G. D., 516, 562
 Williams, J. W., 205, 220
 Williams, M. M. D., 254, 255, 269
 Williams, R. D., 665, 697
 Williams, R. G., 123, 148
 Williams, R. H., 627, 628, 629, 640, 646, 661
 Williams, R. J., 672, 673, 676, 690, 694, 697
 Williams, R. R., 543, 563, 665, 690
 Williams, R. T., 645, 658
 Williamson, M. B., 368
 Williamson, R., 484, 492
 Willis, D. A., 116
 Willmon, T. L., 32, 53
 Wilson, H. D., 87, 119
 Wilson, J. F., 633, 651
 Wilson, J. G., 382, 390
 Wilson, J. M. G., 550, 560
 Wilson, J. R., 242, 382, 390
 Wilson, J. W., 265, 268, 542, 556
 Wilson, R. A., 640, 661
 Wilson, W. C., 200
 Windaus, A., 323, 326, 329, 336, 681, 697
 Winkler, A. W., 105, 114
 Winkler, F., 136, 144, 152
 Winkler, W., 621, 661
 Winsauer, H. J., 630, 661
 Winslow, C., 248, 249, 250, 269
 Winslow, C. E. A., 259, 260, 267
 Winsor, T., 29, 31, 53, 239, 240, 241, 243
 Winter, C. A., 442, 464
 Winter, H., 621, 661
 Winternitz, R., 523, 563
 Wintrobe, M. M., 661, 666, 670, 673, 689, 698
 Wisansky, W. A., 534, 559
 Wise, J. C., 680
 Wislocki, G. B., 186, 189, 190, 191, 192, 193, 435, 447, 464
 Wisemann, M., 210, 219
 Wissler, R. W., 445, 456, 664, 689
 Witten, V. H., 41, 59
 Wittkover, E., 305, 308
 Wittle, E. L., 326, 334
 Woeber, K., 13, 25
 Wöhlisch, E., 5, 7, 8
 Woerdeman, M. J., 448, 462
 Wohlgemuth, J., 472, 477, 482, 526, 563, 575, 579
 Wohnlich, H., 468, 477, 482
 Wohnlich, H. I., 228, 232
 Wolbach, S. B., 382, 390, 443, 464, 606, 661, 670, 680, 698
 Wolf, D. E., 667, 673, 692, 693
 Wolf, H., 107, 118
 Wolf, J., 592
 Wolf, S., 305, 308
 Wolf, S. G., 120, 144, 152
 Wolff, H. G., 120, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 144, 148, 149, 150, 151, 152
 Wolff, S., 41, 43, 47, 54
 Wolkin, J., 88, 114, 164, 165, 195, 270, 283
 Wolman, M., 436, 464

- Wolpers, C., 408, 412, 417, 438, 448, 464
 Womack, M., 368, 390
 Wong, H., 452, 454
 Wood, T. R., 677, 695
 Woodard, G., 40, 54
 Woodard, M., 46, 54
 Woodbury, R. A., 102, 119
 Woodier, A. B., 527, 553
 Woodruff, B. G., 19, 24
 Woods, M., 540, 563
 Woods, M. W., 540, 561
 Woodward, R. B., 329, 336
 Woollard, H. H., 94, 119, 123, 147, 152, 180, 181, 200
 Woolley, D. W., 661, 669, 676, 677, 690, 698
 Worcester, J., 213, 214, 218
 Worcester, L., 265, 268
 Woringer, F., 699, 706
 Wormall, A., 312, 313, 314, 321, 323, 325, 327, 328, 329, 330, 334
 Wortham, J. T., 640, 647
 Wright, C. I., 526, 559
 Wright, C. S., 622, 661, 670, 681, 698
 Wright, L. D., 543, 563
 Wright, P. A., 544, 563
 Wührer, J., 640, 661
 Wurm, M., 549, 560
 Wybauw, L., 90, 119
 Wyckoff, R. W. G., 408, 410, 411, 416, 448, 464
 Wynn, W., 466, 480, 483, 484, 490, 492, 494, 496, 499, 511, 514
 Yaglou, C. P., 260, 269
 Yamada, G., 158, 200
 Yamasaki, Y., 523, 563
 Yannet, H., 498, 500, 502, 512
 Yater, W. M., 105, 119
 Yosikawa, H., 661
 Young, E. G., 641, 642, 643, 661
 Young, M. H. C., 87, 111
 Young, M. W., 630, 661
 Yuditskaya, A. I., 409, 417
 Yuile, C. L., 219
 Yuyama, H., 190, 200, 468, 482
 Zabo, H., 688, 691
 Zahn, H., 351, 358, 362
 Zaiser, E. M., 228, 232
 Zak, F. G., 154, 181, 197, 199, 270, 283, 288, 307, 330, 334
 Zakon, S. J., 298, 302, 308, 626, 657, 685, 697
 Zamecnik, P. C., 573, 579, 704, 707
 Zander, E., 125
 Zappasodi, P., 459
 Zara, M., 628, 649
 Zarafonetis, C. J. D., 542, 563
 Zazeela, H., 102, 104, 105, 109
 Zehender, F., 289, 292, 308, 312, 313, 314, 325, 336
 Zheutlin, H. E. C., 48, 59, 494, 495, 501, 502, 514
 Zieler, 375, 389
 Zierles, K. L., 500, 501, 511
 Zimmerman, H. M., 270, 272, 281, 283
 Zimmermann, K. W., 621, 661
 Zingsheim, M., 352
 Zon, L., 681, 689
 Zopf, L. C., 49, 56
 Zotterman, Y., 12, 21, 24, 61, 118, 136, 152
 Zuckerman, R., 41, 54
 Zuckerman, S., 381, 382, 387, 390, 440, 441, 461, 464
 Zuntz, N., 623, 661
 Zurhelle, E., 639, 661
 Zweifach, B. W., 61, 63, 64, 67, 70, 72, 73, 74, 75, 76, 77, 78, 79, 83, 95, 110, 119, 452, 464, 686, 689, 698
 Zwick, K. G., 43, 59







checked.
msh
12/11

1080

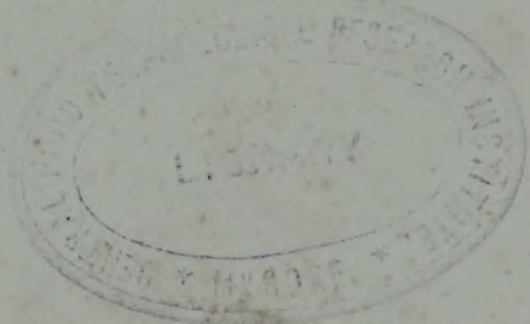
C. F. T. R. I. LIBRARY, MYSORE

21/8/89

10
5.5.97

CHECKED
2008
e

VERIFIED
2013
MS



CFTRI-MYSORE



3304

Physiology and b..



